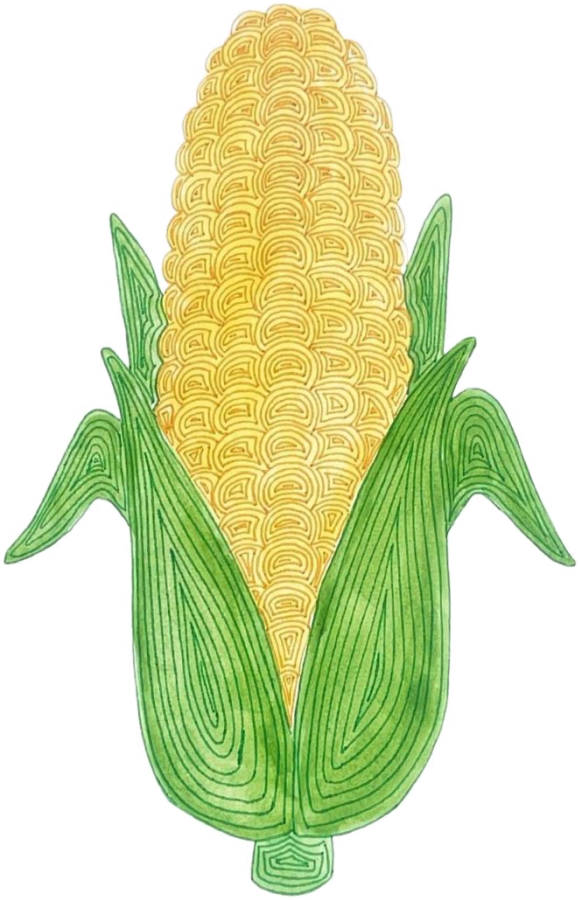
# 67th Annual Maize Genetics Meeting

Program and Abstracts

## March 6 – 9, 2025



MGC

Maize Genetics Cooperation, Inc.

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## This conference received financial support from:

National Science Foundation
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***We thank these sponsors for their generosity!***

A special thank you for the in-kind support from the USDA-ARS.

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**Cover image description**

Zentangle of corn, more pigmented

**Cover art by**

Sarah Fitzsimmons
University of Missouri
USA

# General Information

## Meeting Registration

Thursday: 3:00 PM to 9:30 PM: Depot Registration Office
Friday: 7:30 AM to 12:30 PM: Depot Registration Office

## Meals

All meals will be served buffet style in Midway West and Pegram areas; 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 Midway West, 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The maize meeting is a forum for presentation and discussion of unpublished material.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**.

## Health and Safety Policy

The Maize Genetics Cooperation (MGC) is committed to the health and safety of all Cooperation members and attendees of the Annual Maize Genetics Meeting (MGM). In keeping with the United States Centers for Disease Control (CDC) guidelines, we have developed the following health & safety policy for the 2025 Maize Genetics Meeting. https://www.maizegdb.org/mgc/maizemeeting/2025/health.php

We do not require masks to be worn during the meeting. However, please be sensitive to the immunocompromised and those practicing physical distancing.

If you are feeling unwell, please stay in your hotel room and contact Frankie Palmer.
Frankie's Cell: 402-617-4292

If it is an emergency, call 911.

## Hospitality

After the evening sessions on Thursday and Friday there will be informal socializing and poster gazing in the Midway, with refreshments and games provided from 9 PM - 11 PM and a cash bar until 12 AM. Additionally, from 9 PM - 10:30 PM we will have cornhole boards out for informal play on Thursday, and the initial rounds of a tournament on Friday. On Saturday evening there will be informal socializing in the Midway, with refreshments from 9 PM - Midnight, a cash bar from 11 PM - 12 AM, and the cornhole tournament finals from 10 PM - 12 AM.

## Access to recorded sessions

All talks and sessions will be recorded and made available to each meeting registrant. Registrants will receive an invitation email to view the recordings within 1-2 weeks after the meeting concludes from the Maize Genetics Cooperation (noreply-maize@iastate.edu). If you do not receive the email by March 31st, please check your junk/spam folder. If you still haven't received it, or you are having issues with the site where the videos are hosted, please email john.portwood@usda.gov.

## Steering Committee

Please share your suggestions and comments about the meeting with the 2025 Steering Committee

| | | |
|---|---|---|
| Sherry Flint-Garcia, Chair | (sherry.flint-garcia@usda.gov) | Ex officio: |
| Frank Hochholdinger, co-Chair | (hochhold@uni-bonn.de) | Carson Andorf - MaizeGDB |
| Ruben Rellan-Álvarez, Previous Chair | (rrellan@ncsu.edu) | Erin Sparks - Treasurer |
| Oyenike Adeyemo | (aoadeyemo@unilag.edu.ng) | Darwin Campbell – Planning / Audio Visual |
| Hank Bass | (bass@bio.fsu.edu) | Marty Sachs - Local Host |
| Keting Chen | (kchen@iastate.edu) | John Portwood - Logistics Coordinator |
| Melissa Draves | (madcfr@missouri.edu) | |
| Sarah Jensen | (sarah.jensen@syngenta.com) | Meeting planning: |
| Katie Murphy | (kmurphy@danforthcenter.org) | Tricia Simmons – Conference Direct |
| Cinta Romay | (mcr72@cornell.edu) | Garrett Simmons – Conference Direct |
| Sylvia Morais de Sousa Tinoco | (sylvia.sousa@embrapa.br) | Frankie Palmer – Conference Direct |
| Graziana Taramino | (graziana.taramino@bayer.com) | Stephanie Maher – Conference Direct |
| Feng Tian | (ft55@cau.edu.cn) | |
| Petra Wolters | (petra.wolters@corteva.com) | |


## Acknowledgements

Many thanks go to Carson Andorf, Daniel Kick, John Portwood, the MaizeGDB staff from the USDA-ARS, and Darwin Campbell (Iowa State University) for their tremendous efforts in organizing, assembling, and advertising the conference program. We also greatly thank Tricia Simmons, Garrett Simmons, Frankie Palmer, and their team at ConferenceDirect for helping to organize and implement the conference registration platform, handling meeting logistics with the venue staff, and dealing with many other issues. Special thanks are also extended to the Union Station staff for their help in organizing this conference. Thanks go to Sarah Jensen, Graziana Taramino, and Petra Wolters for their efforts in securing funding to offset meeting costs. Finally, many, many thanks go to the Steering Committee for organizing the 67th Maize Genetics Meeting

# From the Maize Genetics Cooperation Board of Directors

## Maize Genetics Awards:




**The 2025 MGC Cooperator Awardees**

**Don McCarty**, University of Florida
**Karen Koch**, University of Florida
**Frank Hochholdinger**, University of Bonn
**Caroline Macron**, University of Bonn

**The 2025 MGC Leadership Awardee**

**Shawn Kaeppler**, University of Wisconsin

**The 2025 M. Rhoades Early Career Awardee**

**Erin Sparks,** University of Missouri

**The 2025 L. Stadler Mid-Career Awardee**

**Sherry Flint-Garcia**, USDA Agricultural Research Service


**The 2025 R. Emerson Lifetime Awardees**

**Brian Larkins,** University of Nebraska, passed away on January 19th.
**Zac Cande,** University of California Berkley, now retired

## The Barbara McClintock Prize for Plant Genetics and Genome Studies

This award 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 **2025** Barbara McClintock Prize for Plant Genetics and Genome Studies has been awarded to Dr. Edward S. Buckler IV of United States Department of Agriculture - Agricultural Research Service (USDA-ARS) who will present a McClintock Prize Address on Friday, March 7, 8:20 pm CDT (See Page 36).

(See https://maizegdb.org/mgc/awards/ for details about each award)

## NSF-funded support for maize genetics:

The National Science Foundation is supporting the 66th-68th Annual Maize Genetics Meetings with a grant awarded on November 14th, 2023 (IOS-2329928). This conference grant will support, Enhancing Institutional Access (EIA), and Disciplinary Breadth (DB) award programs. These programs broaden the participation of historically marginalized researchers with an interest in exploring research possibilities in maize genetics by providing funding to attend the meeting. These travel participants are paired with a team of mentors that included an early career scientist, an academic PI, and an industry or government scientist that engage with the awardees formally and informally before, during, and after the meeting. Throughout the meeting participants receive professional development training, and opportunities to engage directly with leaders in the field through organized meals with invited speakers. These experiences are vital catalysts for the careers of aspiring student and postdoc trainees. 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.

In addition, the maize genetics community participates in the ROOT & SHOOT RCN project (DBI-2134321). The ROOT & SHOOT group of seven plant science organizations was awarded a five-year grant through the NSF's LEAPS [LEAding cultural change through Professional Societies (LEAPS) of Biology] program. This Research Coordination Network (RCN) includes American Phytopathological Society (APS), American Society of Plant Biologists (ASPB), American Society of Plant Taxonomists (ASPT), Botanical Society of America (BSA), International Society for Molecular Plant Microbe Interactions (IS-MPMI), Maize Genetics Cooperation (MGC), North American Arabidopsis Steering Committee (NAASC), as well as other organizations that serve marginalized scientists.
Two first-time attendees have been supported by ROOT & SHOOT to attend the 2025 meeting.

# The 2025 Attendance/Travel Awards Program

The maize community offers several attendance and travel awards to encourage attendance at the Annual Maize Genetics Meeting (MGM). There are several programs that provide travel/attendance awards; e.g. Disciplinary Breadth (DB), Expanding Institutional Access (EIA), and a general program to support first time attendees. The Maize Genetics Meeting also partners with the ROOT & SHOOT (R&S) Research Coordination Network to allow two first-time attendees to attend the meeting.

Here are the awardees for 2025; please congratulate these scientists and welcome them to our famously hospitable conference!

**Undergraduate Student**

Daisy Montero Ibarra, University of California - Davis
Maria Ximena Anleu Gil, UCD, Postdoctoral Mentor

**Graduate Student**

| | |
|---|---|
| Mariana Chavez, Montclair State University | Poster # 22 |
| Enameguono Olomuko, Purdue University | Poster # 34 |
| Sara Hafiza, Florida State University | Short Talk [T23] |
| Hank Bass, FSU, Faculty Mentor | Poster # 87 |
| Sebastian Mueller, Oregon State University | Poster # 158; Lightning Talk [L5] |
| Edward Fernandez, North Dakota State University | Poster # 174 |
| Hanna Pil, North Carolina State University | Poster # 202; Lightning Talk [L8] |
| Vitor Sagae, University of Florida | Poster # 238 |
| Shreejana KC, University of Delaware | Poster # 256 |

**Faculty**

| | |
|---|---|
| Gwonjin Lee, West Virginia State University | Poster # 15; Lightning Talk [L28] |
| Beth Thompson, East Carolina University | |
| Veronica Justen, University of Wisc. - River Falls | Poster # 282 |

The Maize Genetics Meeting is supported in part by IOS Award #2329928 from the National Science Foundation, Plant Genome Research Program.

# Broadening International Participation Awards

The 2025 Broadening International Participation Award program seeks to promote international attendance for researchers from countries that are historically under-represented at the Maize Meeting. This 2025 award program seeks to enrich the maize community and broaden the opportunities to learn about maize genetics by connecting with scientists in the maize genetics community, exploring potential collaborations, and developing career contacts. BIP awardees receive waived registration to the recorded talks and sessions.

**Research Scientists**
Elena Andriunaite

**Postdoc**
Muhammad Zafar Iqbal

**Graduate Student**
Md Nazmul Hossain

# FAIR Data Management

*…A Reminder from the MaizeGDB team*


MaizeGDB is a founding member of the AgBioData Consortium. AgBioData is a consortium of agricultural biological databases with the mission of consolidating standards and best practices for acquiring, displaying, and reusing genomic, genetic, and breeding (GGB) data.

Member databases of the AgBioData consortium follow the Findable, Accessible, Interoperable, and Reusable (FAIR) principles, enhancing the accessibility and reusability of large-scale agricultural research data. MaizeGDB consolidates vast amounts of published data, simplifying discovery and utilization for our community. Your commitment to FAIR principles (go-fair.org) enables MaizeGDB and similar platforms to integrate and leverage even more data efficiently. Below are essential guidelines for applying FAIR data management practices to your generated data, as well as to the data you encounter in research papers and grant reviews.

- **Database Selection and Accession Reporting:** Ensure your data, such as DNA/RNA/Protein sequences, genome assemblies, and annotations, are submitted to long-term repositories like NCBI. Always include accession numbers in your publication. For maize SNPs, submit them to EVA at EBI. Explore more repositories at maizegdb.org/FAIRpractices and consult journal guidelines for additional instructions.

- **Data Publication:** Publish your data concurrently with your paper. For datasets not included with the article, secure a persistent identifier (e.g., DOI) from the data repository to reference in your paper. Datasets can be independently published in journals like microPublication, ensuring they are linked to the corresponding paper. Verify the presence and FAIR compliance of reported data during peer review.

- **Gene and Protein Identifier Usage:** Use established identifiers for genes, gene models, and genomes. Avoid renaming existing genes. Look up gene symbols at MaizeGDB and use precise gene model IDs. For protein data, reference the correct ID from NCBI or UniProt, submitting new sequences to these repositories as necessary. Maize nomenclature guidelines: https://www.maizegdb.org/nomenclature

- **Metadata and File Format Standards:** Attach comprehensive metadata to your datasets and adhere to accepted file formats. Treat metadata with the same rigor as experimental and analysis work. Incomplete or poorly described datasets compromise reusability, reproducibility, and overall research quality.

- **Machine-Readable Data Sets:** Ensure your data is machine-readable, using permanent identifiers and correct terminology (e.g., using correct case for genetic loci, incorporating GO, PO, PATO terms). Validate your data against common, established machine-readable formats.

- **Data Management Planning:** Allocate sufficient time for meticulous data management, similar to the effort dedicated to other research aspects.

- **FAIR Data Standards:** Here are some resources: https://www.go-fair.org, https://doi.org/10.1093/database/bay088.

**We are always happy to answer your questions on these issues!** https://www.maizegdb.org/contact

## What's NEW at MaizeGDB!

In 2024, MaizeGDB expanded its pan-genomic resources* for the representative B73 genome, all NAM founder lines, Pan-Andropogoneae, and other historically important lines. These resources now include:

- 110 genomes, two are under the Toronto agreement, including the Pan-Andropogoneae genomes
- Over 1.5 million new gene model annotations
- New Pan-gene dataset, which includes 57 annotations
- Hundreds of new downloadable files
- 391 target databases in BLAST
- Syntenome and reelGene tracks on the B73v5 genome browser
- 400+ high-throughput sequencing data for over 80 tissues/conditions
- 300+ traits linked to over 40,000 positions in the genome
- 80+ million SNPs from EVA and Ensembl Plants
- Genotype data remapped to B73 v5 for 1,500 maize accessions
- Over 1 million predicted GO terms across 31 genomes
- Resources for 4 insertion mutation collections
- MaizeMine has been updated to include B73_v5 and the NAM founder lines
- SNPversity 2.0, which enables users to explore and visualize extensive variant datasets with ease**
- Transposable elements, structural variation, regulatory sites, and more…

**If you have questions on how to access/use these resources, contact us at**
https://www.maizegdb.org/contact

*Cannon, EK et al. (2024) Enhanced Pan-Genomic Resources at the Maize Genetics and Genomics Database. Genetics. doi: https://doi.org/10.1093/genetics/iyae036.

**Andorf CM et al. (2024) A unified VCF data set from nearly 1,500 diverse maize accessions and resources to explore the genomic landscape of maize. G3. doi: https://doi.org/10.1093/g3journal/jkae281

**Thank you to the 2024 MaizeGDB Editorial Board Members!**
Amruta Bapat, Washington University St. Louis
Hank Bass, Florida State University
Diana Escamilla Sanchez, Iowa State University
Rohit Kumar, Clemson University
Songyu Liu, China Agricultural University, China
Ankita Mishra, Orissa University of Agriculture and Technology, India
Aimee Uyehara, Stanford University (2nd Year!)

## MaizeGDB has partnered with *microPublication Biology!*

***microPublication Biology* (Caltech Publishers) is a peer-reviewed, open-access journal that publishes single experiment results, which are incorporated directly into community knowledgebases like MaizeGDB**!

*microPublication Biology* gets your individual research findings, that might otherwise remain unpublished, out to the scientific community while providing credit to those who did the work. Articles are small (one figure, few pages), peer-reviewed, assigned a DOI and are discoverable on PMC, PubMed, EuropePMC, and Google Scholar.

How it works: Each maize *microPublication Biology* submission will be vetted by MaizeGDB curators at the time of peer review to ensure data meets FAIR data standards. Upon acceptance, your article is curated into MaizeGDB which couples the publication with curation and discoverability in MaizeGDB. The cost to publish is only $350.

**Here are some recent maize publications:**

Gustin JL, Zimmerman SA, and Sachs MM. (2025) Allelism of Uncharacterized Dwarf Mutants in Maize. https://doi.org/10.17912/micropub.biology.001504

Reneau, J; Ouslander, N; Sparks, EE. (2024) Quantification of maize brace root formation after vertical stalk displacement. microPublication Biology. https://doi.org/10.17912/micropub.biology.001189

Linders, KM; Santra, D; Schnable, JC; Sigmon, B. (2024) Variation in Leaf Chlorophyll Concentration in Response to Nitrogen Application Across Maize Hybrids in Contrasting Environments. microPublication Biology. https://doi.org/10.17912/micropub.biology.001115

**For more information:**
Visit the journal: https://www.micropublication.org

Questions can be sent to:
Karen Yook, Executive Editor (karen.yook@micropublication.org)
Carson Andorf, Maize Science Officer (carson.andorf@usda.gov)

# SCHEDULE OF EVENTS

## Talks will be held in the Grand Ballroom
## Posters will be displayed in Midway West

### Wednesday, March 5, 2025

| | | |
|---|---|---|
| 8:30 AM – 6:00 PM | **OPTIONAL PRE-CONFERENCE WORKSHOPS** | |
| 8:30 AM - 11:30 AM | **Maize Crop Germplasm Committee** | Missouri History Museum |
| 1:00 PM – 6:00 PM | **Corn Breeding Research (Day 1)** | Midway 1 & 2 |

### Thursday, March 6, 2025

| | | |
|---|---|---|
| 9:00 AM – 6:00 PM | **OPTIONAL PRE-CONFERENCE WORKSHOPS** | |
| 8:00 AM – 5:00 PM | **Corn Breeding Research (Day 2)** | Midway 3 & 4 |
| 9:00 AM - 4:00 PM | **Development & Cell Biology Workshop** | Midway 1 & 2 |
| 9:30 AM - 3:00 PM | **Bayer Tour** | Bus pick-up |
| 12:00 PM - 2:00 PM | **39N Lunch and Danforth Center Tour** | 1001 N. Warson Road<br>St. Louis, MO 63132 |
| 2:00 PM - 5:00 PM | **Computational Resources Workshop** | Conductor Room |
| 3:00 PM – 9:30 PM | **REGISTRATION** | Depot Registration Office |
| 3:00 PM – 6:00 PM | **POSTER HANGING** | Midway West |
| 5:00 PM – 5:45 PM | **Travel awardees / mentors meet & greet** | Jeffersonian /<br>Knickerbocker |
| 6:00 PM – 7:00 PM | **DINNER** | Midway West & Pegram |

## Thursday, March 6, 2025 (continued)

| | | |
|---|---|---|
| 7:00 PM – 9:00 PM | **SESSION 1 – WELCOME / KEYNOTE/ GENE REGULATION**<br>Chair: Sherry Flint-Garcia | |
| 7:00 PM | **WELCOME AND ANNOUNCEMENTS** | Grand Ballroom |
| 7:20 PM | **Jonathan Wendel, Iowa State University**<br>*Genes, jeans, genomes, and the wonders of polyploidy in plants* | [KS1] |
| 8:00 PM - 8:20 PM | **POSTER LIGHTNING TALKS** | |
| | **Yue Liu, Iowa State University**<br>*Candidate Genes Underlying a Major QTL qshgd1 Causing Spontaneous Haploid Genome Doubling in Maize A427* | [L1, P135] |
| | **Vladimir Torres-Rodriguez, University of Nebraska-Lincoln**<br>*Multi-species transcriptome-wide association studies identify additional genes controlling flowering* | [L2, P250] |
| | **Andrea Sama, University of California, San Diego**<br>*Chemical Imaging Reveals Metabolic Responses to Salt-Stress in Maize Roots* | [L3, P84] |
| | **Xiaosa Xu, University of California, Davis**<br>*A high-resolution, meristem stage-specific single-cell gene expression atlas resolving developmental dynamics in maize inflorescence architecture* | [L4, P75] |
| | **Sebastian Mueller, Oregon State University**<br>*Predictive Modeling of Pollen Fitness Phenotypes from Genome Scale Data Identifies Expression Specificity As a Critically Informative Parameter* | [L5, P158] |
| | **Huan Chen, Michigan State University**<br>*Archaeological Bolivian maize genomes suggest Inca cultural expansion augmented maize diversity in South America* | [L6, P133] |
| | **Lukas Würstl, Technical University Munich**<br>*Natural alleles of the gene lhcb6 shape photosynthesis and key agronomic traits in maize (Zea mays L.) landraces* | [L7, P252] |
| | **Hannah Pil, North Carolina State University**<br>*BZea: A diverse teosinte introgression population for improving modern maize sustainability* | [L8, P202] |

## Thursday, March 6, 2025 (continued)

| | | |
|---|---|---|
| 8:20 PM | **Maike Stam, University of Amsterdam**<br>*Vgt1 as enhancer of ZmRap2.7 impacts flowering time and gene regulatory networks involved in jasmonate signaling in maize* | [T1] |
| 8:40 PM | **Ankush Sangra, University of Georgia**<br>*Decoding a complex distal non-coding QTL at TEOSINTE BRANCHED 1* | [T2] |
| 9:00 PM – 12:00 AM | **INFORMAL POSTER VIEWING & HOSPITALITY** | Midway West |
| 9:00 PM – 10:30 PM | **INFORMAL CORN HOLE PLAY** | Regency Ballroom |

## Friday, March 7, 2025

| | | |
|---|---|---|
| 7:00 AM – 8:00 AM | **BREAKFAST** | Midway West |
| 7:30 AM – 12:30 PM | **REGISTRATION** | Depot Registration Office |
| 8:15 AM – 10:15 AM | **SESSION 2 – MODELING CORN**<br>Chair: Hank Bass | |
| 8:15 AM | **ANNOUNCEMENTS** | Grand Ballroom |
| 8:30 AM | **Jingjing Zhai, Cornell University**<br>*Cross-species modeling of plant genomes at single nucleotide resolution using a pre-trained DNA language model* | [T3] |
| 8:50 AM | **Diana Ruggiero, Oregon State University**<br>*Quantitative genetics of leaf vascular density in maize* | [T4] |
| 9:10 AM | **Erin Farmer, Cornell University**<br>*Integrating proximal sensing modalities for enhanced prediction of agronomically important crop traits* | [T5] |
| 9:30 AM | **Lucas Batista & Joseph Gage, Kansas State University & USDA-ARS**<br>*Crowdsourcing phenotype prediction: Results from the 2024 G2F prediction competition.* | [T6] |
| 9:50 AM - 10:15 AM | **POSTER LIGHTNING TALKS** | |
| | **Jacob Kelly, University of Missouri**<br>*Speed Breeding Fast-Flowering Mini-Maize* | [L9, P268] |
| | **Thanduanlung Kamei, University of Delaware**<br>*SBP mutants have an expanded competence zone for brace root initiation* | [L10, P110] |
| | **Katy Guthrie, University of Minnesota**<br>*Teaching Scientific Writing Alongside the Scientific Method in an Introductory Plant Biology Lab* | [L11, P190] |
| | **Joseph DeTemple, Iowa State University**<br>*Gene expression and circadian rhythm differences between temperate and tropical maize inbreds in response to photoperiod* | [L12, P41] |

## Friday, March 7, 2025 (continued)

| | | |
|---|---|---|
| | **Manisha Munasinghe, University of Minnesota**<br>*Structural Variation has a Limited Role in Influencing Genome-Wide Differential Gene Expression Patterns in Maize* | [L13, P16] |
| | **Mohamed El-Walid, Cornell University**<br>*Genomic Assembly and Analysis of Fast-Flowering Mini-Maize* | [L14, P144] |
| | **Christopher Benson, Ohio State University**<br>*Resolving Maize Domestication and Subpopulation Divergence Using Long Terminal Repeat Retrotransposons* | [L15, P13] |
| | **Xuelian Du, University of Bonn**<br>*BonnMu – A resource for functional genomics in maize (Zea mays L.)* | [L16, P289] |
| 10:15 AM - 10:45 AM | **BREAK** | Prefunction Space<br>Grand Ballroom |
| 10:45 AM – 12:15 PM | **SESSION 3 – EDUCATION, COMMUNITY, AND OUTREACH**<br>Chair: Brandi Sigmon | |
| 10:45 AM | **ACKNOWLEDGE TRAVEL AWARDEES** | Grand Ballroom |
| 10:55 AM - 11:10 AM | **POSTER LIGHTNING TALKS** | |
| | **Jason Lynn, Cold Spring Harbor Laboratory**<br>*AGO2 and AGO3 regulate RNAi fidelity by suppressing RNA-directed DNA methylation* | [L17, P285] |
| | **Dafang Wang, Hofstra University**<br>*Mechanisms of Small RNA-Induced Epigenetic Silencing of Ac Transposons in Maize* | [L18, P299] |
| | **Vinay Chaudhari, Donald Danforth Plant Science Center**<br>*Predicting end-of-season Sorghum biomass from seedling-stage traits* | [L19, P265] |
| | **Olivia Haley, USDA-ARS, ORISE**<br>*Comparing the performance of protein folding models AlphaFold, ESMFold, and Boltz for classical genes in maize* | [L20, P129] |
| | **Zong-Yan Liu, Cornell University**<br>*ReelGene2: A Large Language Model for Single Base Pair Precision Gene Annotation in Diverse Plant Genomes* | [L21, P160] |

## Friday, March 7, 2025 (continued)

| | | |
|---|---|---|
| 11:10 AM | **John Fowler, Updates from RCN, Resources** | [T7] |
| 12:15 PM - 1:15 PM | **LUNCH**<br>Special table at lunch w/ Helen Anne Curry | Midway & Pegram |
| | Travel Awardee/Mentor Networking Lunch | Midway 1 & 2 |
| | MGC BoD and MGAC Lunch | Midway 3 & 4 |
| 1:30 PM - 4:30 PM | **POSTER SESSION 1** | Midway West |
| 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 West

| | | |
|---|---|---|
| 4:40 PM – 6:00 PM | **SESSION 4 – MAIZE UNDER STRESS**<br>Chair: Melissa Draves | |
| 4:40 PM | **Veronica Perez, Cornell University**<br>*Translational and proteomic analysis of cold-stressed maize reveals ribosomal protein families involved in cold response and tolerance* | [T8] |
| 5:00 PM | **Fausto Rodríguez-Zapata, North Carolina State University**<br>*Introgression of a Mexican highland chromosomal inversion into temperate maize accelerates flowering, promotes growth, and modulates a cell proliferation gene network.* | [T9] |
| 5:20 PM | **Marie-Laure Martin, INRAE**<br>*Integration of phenomic, proteomic, and genomic data into a multi-scale network unravels missing heritability for maize response to water deficit* | [T10] |
| 5:40 PM | **Maggie Woodhouse, USDA-ARS (Cancelled)**<br>*Transcriptional regulation of stress adaptation in maize: identification and functional annotation* | [T11] |

## Friday, March 7, 2025 (continued)

| | | |
|---|---|---|
| 6:00 PM - 7:00 PM | **DINNER** | Midway & Pegram |
| | Bayer Student/Postdoc Dinner | Midway 1 & 2 |
| 7:00 PM –9:00 PM | **SESSION 5 – AWARDS**<br>Chair: Andrea Eveland | |
| 7:00 PM | **Andrea Eveland**<br>*Introduction to Awards* | Grand Ballroom |
| 7:10 PM | **Natalia de Leon (Matt Hufford substituting)**<br>*Presenting: Cooperator and Leadership Awards* | |
| 7:30 PM | **Andrea Eveland**<br>*Presenting: M. Rhoades Early-Career, L. Stadler Mid-Career* | |
| 7:50 PM | **Andrea Eveland and Wojtek Pawlowski**<br>*Presenting: R. Emerson Lifetime Awards* | |
| 8:10 PM | **Marna Yandeau-Nelson**<br>*McClintock Prize Presentation Introduction* | |
| | **Edward Buckler IV, USDA Agricultural Research Service (Cancelled)**<br>*Why do we do maize genetics?* | [M1] |
| 8:20 PM | **Helen Anne Curry, Georgia Tech**<br>*Input, Insurance, Objective: Reflections on diversity from the history of crop science* | [KS2] |
| 9:00 PM - 12:00 AM | **INFORMAL POSTER VIEWING & HOSPITALITY** | Midway West |
| 9:30 PM - 10:00 PM | **OPERA BELL BAND** | Pegram |
| 9:00 PM - 10:30 PM | **EARLY BRACKETS OF THE CORN HOLE TOURNAMENT** | Regency Ballroom |

## Saturday, March 8, 2025

| | | |
|---|---|---|
| 7:00 AM – 8:00 AM | **BREAKFAST** | Midway West & Pegram |
| 8:00 AM – 12:00 PM | **REGISTRATION** | Depot Registration Office |
| 8:15 AM – 10:00 AM | **SESSION 6 – ROOTS & NUTRIENT UPTAKE / KEYNOTE**<br>Chair: Rubén Rellán Álvarez | |
| 8:15 AM | **ANNOUNCEMENTS** | Grand Ballroom |
| 8:20 AM | **Alexander Liu, Washington University in Saint Louis, Donald Danforth Plant Science Center**<br>*A Rootless1 knockdown allele affects maize nodal root development, increasing rooting depth, nitrogen uptake efficiency, and grain production in the field* | [T12] |
| 8:40 AM | **Sylvia Morais de Sousa Tinoco, Embrapa**<br>*Overexpression of PSTOL1-like genes increases maize root surface area and biomass under low and high phosphorus conditions* | [T13] |
| 9:00 AM | **Ivan Baxter, Donald Danforth Plant Science Center**<br>*You need a real maize geneticist* | [KS3] |
| 9:40 AM – 10:00 AM | **POSTER LIGHTNING TALKS** | |
| | **Forrest Li, University of California, Davis**<br>*Sequencing a seed bank: Assessing the utility of environmental data from CIMMYT traditional varieties for climate-adaptive maize breeding* | [L23, P267] |
| | **Aimee Schulz, University of Minnesota**<br>*The molecular evolution of perenniality across the grasses* | [L24, P20] |
| | **Matthew Wendt, Iowa State University**<br>*Environmental and Genetic Factors Underlying Maize Cuticular Wax Accumulation Under Drought Stress* | [L25, P220] |
| | **Wen-Yu Liu, North Carolina State University**<br>*ZmCER9-Mediated Regulation of Autoactive NLR Proteins and Effector-Triggered Immunity via ERAD Pathway* | [L26, P72] |

## Saturday, March 8, 2025 (continued)

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|---|---|---|
| | **Huda Ansaf, University of Missouri-Columbia**<br>*Understanding the Role of TOR Signaling and Translational Machinery in Regulating Protein-bound Amino Acid Homeostasis in Maize Kernels* | [L27, P70] |
| | **Gwonjin Lee, West Virginia State University**<br>*Sex-specific patterns of meiotic recombination are determined by maize lines from different climate zones.* | [L28, P15] |
| | **Michelle Stitzer, Cornell University**<br>*Comparative grass genomics reveals explosive genome evolution in maize and its wild relatives* | [L29, P4] |
| | **Mohammad Mahmood Hasan, University of Florida**<br>*mop1 reshapes recombination landscapes by altering DNA methylation and chromatin states at MITEs* | [L30, P303] |
| 10:00 AM – 10:30 AM | **BREAK** | Prefunction Space<br>Grand Ballroom |

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|---|---|---|
| 10:30 AM – 12:30 PM | **SESSION 7 – KEYNOTE / CELL DIVISION & MERISTEMS**<br>Chair: Sarah Jensen | |
| 10:30 AM | **Sióbhan Brady, Howard Hughes Medical Institute, University of California Davis**<br>*Environmental integration with root cell type development* | [KS4] |
| 11:10 AM | **Stephanie Martinez, University of California, Riverside**<br>*Delayed divisions and cell elongation defects influence plant growth in katanin mutants* | [T14] |
| 11:30 AM | **Fang Xu, Shandong University**<br>*The EPF-ERECTA ligand-receptor pairs regulate maize shoot and inflorescence architecture in coordination with CLAVATA pathway in maize.* | [T15] |
| 11:50 AM | **Thu Tran, Cold Spring Harbor Laboratory**<br>*Catalytic and non-catalytic TREHALOSE-6-PHOSPHATE SYNTHASES (TPSs) interact with RAMOSA3 to control maize development* | [T16] |

## Saturday, March 8, 2025 (continued)

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|---|---|---|
| 12:10 PM | **Alejandro Aragon Raygoza, Iowa State University**<br>*Exploring the effects of ethylene-related transcription factors during maize shoot development* | [T17] |
| 12:30 PM - 1:30 PM | **LUNCH** | Midway & Pegram |
| | *Travel awardee lunch with keynote speakers* | Midway 1 & 2 |
| | *Maize genetics mentoring & networking lunch* | Midway 3 |
| | *MGMSC lunch* | Midway 4 |
| 1:30 PM - 4:30 PM | **POSTER SESSION 2** | Midway West |
| 1:30 PM - 3:00 PM | *Presenters should be at even-numbered posters* | |
| 3:00 PM - 4:30 PM | *Presenters should be at odd-numbered posters* | |

Beverages will be available from 2:30 to 4:00 PM in Midway West

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|---|---|---|
| 4:30 PM - 6:00 PM | **COMMUNITY SESSION**<br>**Maize Genetics Cooperative**<br>*Wojtek Pawlowski, MGC BoD Chair* | Grand Ballroom |
| 6:00 PM - 7:00 PM | **DINNER** | Midway West & Pegram |
| | *Corteva Student/Postdoc Dinner* | Midway 1 & 2 |

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|---|---|---|
| 7:00 PM – 8:20 PM | **SESSION 8 – REPRODUCTION / KEYNOTE**<br>Chair: Cinta Romay | |
| 7:00 PM | **ANNOUNCEMENTS** | Grand Ballroom |
| 7:05 PM | **Xixi Zheng, University of Regensburg**<br>*Understanding the Molecular Mechanism of Parthenogenesis in Cereals* | [T18] |
| 7:25 PM | **Rachel Egger, Syngenta**<br>*Heat treatment and UBA2 fusions enhance LbCas12a genome editing activity during haploid induction* | [T19] |
| 7:45 PM | **Elli Cryan, University of California Davis**<br>*Molecular evolution of the Ga reproductive barriers in maize and related species* | [T20] |

## Saturday, March 8, 2025 (continued)

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|---|---|---|
| 8:05 PM | **Doreen Ware, USDA Agricultural Research Service (Cancelled)**<br>*Plant genomes: Understanding their past and managing their future* | [KS5] |
| 8:15 PM - 12:00 AM | **INFORMAL POSTER VIEWING & HOSPITALITY** | Midway West |
| 10:00 PM - 12:00 AM | **GAME NIGHT / CORN HOLE TOURNAMENT FINALS** | Midway West |

## Sunday, March 9, 2025

| | | |
|---|---|---|
| 7:00 AM - 8:20 AM | **BREAKFAST** | Midway & Pegram |
| 8:25 AM – 10:20 AM | **SESSION 9 – EPIGENETICS**<br>Chair: Katie Murphy | |
| 8:25 AM | **ANNOUNCEMENTS** | |
| 8:30 AM | **Qi Li, University of Tuebingen, Germany**<br>*Long-distance retrotransposons direct variable gene imprinting in maize* | [T21] |
| 8:50 AM | **Xi Cheng, University of Florida**<br>*Deciphering epigenetic and genetic alterations in a DNA methylation mutant through successive generations of self-fertilization in maize* | [T22] |
| 9:10 AM | **Hafiza Sara Akram, Florida State University**<br>*Replication timing uncovers a novel two-compartment arrangement of maize interphase euchromatin* | [T23] |
| 9:30 AM | **Akwasi Yeboah, University of Florida**<br>*Determination of Genetic and Epigenetic Regulations of Meiotic Recombination during Domestication in Maize* | [T24] |
| 9:50 AM - 10:20 AM | **BREAK** | |

## Sunday, March 9, 2025 (continued)

| | | |
|---|---|---|
| 10:20 AM – 12:00 PM | **SESSION 10 – BUILDING A STRONGER MAIZE PLANT**<br>Chair: Frank Hochholdinger | |
| 10:20 AM | **Bharath Kunduru, Clemson University**<br>*Deciphering genetic architecture of stalk lodging resistance using high-density phenotype map in maize* | [T25] |
| 10:40 AM | **Laura Tibbs-Cortes, USDA-ARS (Talk Recorded)**<br>*Plasticity and fitness trade-offs in switchgrass revealed by open science and citizen science data* | [T26] |
| 11:00 AM | **Qin Yang, Northwest A&F University**<br>*Inactivation of a lysine-histidine transporter-1 gene confers southern leaf blight resistance in maize* | [T27] |
| 11:20 AM | **Marion Pitz, University of Bonn**<br>*Regulation of heterosis-associated gene expression complementation in maize hybrids* | [T28] |
| 11:40 AM | **CLOSING REMARKS** | |
| 12:00 PM | **ADJOURNMENT** | |

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|---|---|---|
| 1:00 PM – 3:00 PM | **OPTIONAL POST-CONFERENCE WORKSHOP** | |
| 1:00 PM - 3:00 PM | **Missouri Botanical Garden** | Missouri Botanical Garden<br>4344 Shaw Blvd<br>St. Louis, MO 63110 |

# Poster List

## Evolution and Population Genetics

| | | |
|---|---|---|
| *P1* | **Jeffrey Ross-Ibarra** <rossibarra@ucdavis.edu> | *An ancient origin of the naked grains of maize* |
| *P2* | **Adrienne Moran Lauter** <adrienne.moranlauter@usda.gov> | *Analysis of Ga2 genome structure and activity reveals widespread distribution of functional alleles in modern maize germplasm* |
| *P3* | **Gretta Buttelmann** <glb28@iastate.edu> | *Comparative genomic analysis of maize and its wild relatives to identify loci underlying cold tolerance and nitrogen recycling* |
| *P4* | **Michelle Stitzer** <mcs368@cornell.edu> | *Comparative grass genomics reveals explosive genome evolution in maize and its wild relatives* |
| *P5* | **Akwasi Yeboah** <akwasiyeboah@ufl.edu> | *Determination of genetic and epigenetic regulations of meiotic recombination during domestication in maize* |
| *P6* | **Mingyu Wang** <mw36149@uga.edu> | *Discovering mechanisms of maize abnormal chromosome 10 (Ab10) meiotic drive through comparative genomics* |
| *P7* | **Samantha Snodgrass** <ssnodgrass@ucdavis.edu> | *Does maize diversity mirror human diversity across the Americas?* |
| *P8* | **Arnaud Ronceret** <arnaud.ronceret@ibt.unam.mx> | *Evolution of the protein complex involved in early recombination in plants* |
| *P9* | **Amy Pollpeter** <amyrp@iastate.edu> | *Evolutionary determinants of gene expression in maize* |
| *P10* | **Charles Hale** <coh22@cornell.edu> | *Extensive modulation of a conserved cis-regulatory code across 625 grass species* |
| *P11* | **Lina Lopez-Corona** <llopezc@ncsu.edu> | *Genetic and phenotypic characterization of wild teosinte alleles introgressed into elite maize line CML311* |
| *P12* | **Heather Chamberlain** <heather.chamberlain.irwin@univie.ac.at> | *Genetic legacies of the first ancient South American state in archaeological maize* |
| *P13* | **Christopher Benson** <benson.140@osu.edu> | *Resolving maize domestication and subpopulation divergence using long terminal repeat retrotransposons* |
| *P14* | **Sai subhash Mahamkali VS** <mahankalisubhash03@gmail.com> | *Revealing the genetic architecture for different nitrogen responses in sorghum* |
| *P15* | **Gwonjin Lee** <gwonjin.lee@wvstateu.edu> | *Sex-specific patterns of meiotic recombination are determined by maize lines from different climate zones.* |
| *P16* | **Manisha Munasinghe** <mmunasin@umn.edu> | *Structural variation has a limited role in influencing genome-wide differential gene expression patterns in maize* |
| *P17* | **Yuchu Ma** <nathanchu@huskers.unl.edu> | *The effect of modern breeding on rhizosphere microbiome under different nitrogen conditions in maize* |
| *P18* | **Sheng-Kai Hsu** <sh2246@cornell.edu> | *The genetic basis of environmental adaptation in grasses* |

*P19* **Jonathan Acosta** <jonathan.acosta@cinvestav.mx> — *The influence of heavy metal stress 1 on the evolutionary transition of teosinte to maize*

*P20* **Aimee Schulz** <schu4332@umn.edu> — *The molecular evolution of perenniality across the grasses*

## Biochemical and Molecular Genetics

*P21* **Emily Wheeler** <eamarkha@ncsu.edu> — *A modified replication timing profiling method improves early replication detection and facilitates comparison of multiple genotypes*

*P22* **Mariana Chavez-Carranza** <mchavezc03@hotmail.com> — *Annotating of active LTR retrotransposons in course-based genomics research*

*P23* **Sylvia Morais de Sousa Tinoco** <sylvia.sousa@embrapa.br> — *Bacterial strains isolated from Vellozia spp. promote maize growth*

*P24* **Madison Lane** <mlane@iastate.edu> — *Building a synthetic cuticle: Characterizing the impact of maize transcription factors on cuticle biosynthetic pathways*

*P25* **Noah Walberg** <nwalberg@iastate.edu> — *Characterization of transporter genes and their impact on cuticular wax composition on maize silks*

*P26* **Zhijie Shui** <zhijie_shui@nwafu.edu.cn> — *Co-expression analysis identifies a transcription factor positively regulates the kauralexin biosynthesis gene ZmKSL2*

*P27* **Shivreet Kaur** <skaur7@ncsu.edu> — *Comparative analysis of host specificity and pathogen interaction of Cochliobolus heterostrophus on maize vs. non-host species*

*P28* **Bharath Kunduru** <bkundur@clemson.edu> — *Deciphering genetic architecture of stalk lodging resistance using high-density phenotype map in maize*

*P29* **Sylvie Coursol** <sylvie.coursol@inrae.fr> — *Deciphering the role of the cell wall in mycorrhizal symbiosis under low-input conditions in maize*

*P30* **Ankush Sangra** <ankush.sangra@uga.edu> — *Decoding a complex distal non-coding QTL at TEOSINTE BRANCHED 1*

*P31* **Gerardo Gonzalez** <ggg7@hawaii.edu> — *Developing a high-throughput genotyping assay for rapid identification of multiple edited flowering genes in tropical maize*

*P32* **Jane Mascarenhas** <mjane@ksu.edu> — *Developing a system to elucidate genetic factors underlying hypersensitivity responses in maize*

*P33* **Rina Carrillo** <rinamc@hawaii.edu> — *Developing an efficient workflow for detecting CRISPR-edited alleles in three target genes that regulate photoperiod response in tropical maize*

*P34* **Enameguono Olomukoro** <eolomuko@purdue.edu> — *Development of Sorghum BTx642 EMS population*

*P35* **Zhengzhi Zhang** <zhangzheng@umsystem.edu> — *Development of efficient haploid inducers of fast flowering mini maize*

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| *P36* | **Hui Liu**<br><huil@ksu.edu> | *Endogenous gene activation of a MYB transcription factor by CRISPRa drives wax biosynthesis regulation in maize* |
| *P37* | **Cesar Xavier**<br><cdinizx@ncsu.edu> | *Engineering maize mosaic virus for efficient virus induced gene silencing and virus induced DNA-free genome editing in maize: a potential ally for maize improvement* |
| *P38* | **Justin Larkin**<br><jtlarkin@illinois.edu> | *Expression of the solanaceous immune receptor FLS3 in maize protoplasts* |
| *P39* | **Maruti Nandan Rai**<br><mnrai@illinois.edu> | *Expression variation in Glossy15 is associated with harvest index in maize* |
| *P40* | **Zachary Gorman**<br><zachary.gorman@usda.gov> | *Flood-induced insect resistance in maize involves flavonoid-dependent salicylic acid induction* |
| *P41* | **Joseph DeTemple**<br><josephdetemple@gmail.com> | *Gene expression and circadian rhythm differences between temperate and tropical maize inbreds in response to photoperiod* |
| *P42* | **Olga Zimina**<br><oz32@cornell.edu> | *Generating crossovers at targeted sites in the maize genome* |
| *P43* | **Elliot Braun**<br><braunell@msu.edu> | *Genetic architecture of specialized metabolites in sorghum and maize* |
| *P44* | **Hui Jiang**<br><hjiang@danforthcenter.org> | *Harnessing male sterility to accelerate genetic research in the C4 model Setaria viridis* |
| *P45* | **Joerg Degenhardt**<br><joerg.degenhardt@pharmazie.uni-halle.de> | *How maize eliminates an apparent toxic by-product - Identification and functional characterization of the C4-ketone products of homoterpene biosynthesis in Zea mays* |
| *P46* | **Mateusz Zelkowski**<br><mz548@cornell.edu> | *How to control meiotic recombination: The role of chromatin state and DSB resection in determining crossing-over locations in maize* |
| *P47* | **Sylvia Morais de Sousa Tinoco**<br><sylvia.sousa@embrapa.br> | *Impact of phosphate-solubilizing bacteria inoculation on maize yield, root system architecture and microbiome* |
| *P48* | **Zackariah Ellington**<br><zfleming3@unl.edu> | *Implementation of a lab-scale highly efficient CRISPR cas-9 gene editing and genotyping system in maize.* |
| *P49* | **Fausto Rodríguez-Zapata**<br><frodrig4@ncsu.edu> | *Introgression of a Mexican highland chromosomal inversion into temperate maize accelerates flowering, promotes growth, and modulates a cell proliferation gene network.* |
| *P50* | **Ana Lúcia Pinheiro**<br><anampinh@gmail.com> | *LYSDH overexpression increases drought tolerance in maize* |
| *P51* | **Jonathan Gent**<br><gent@uga.edu> | *Methylation-responsive promoters for engineering gene expression* |
| *P52* | **Caroline Henry**<br><chenry@danforthcenter.org> | *Non-invasive identification and analysis of maize root exudates* |

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| *P53* | **Megan DeTemple** <mf6130@iastate.edu> | *Novel use of cell wall degrading enzymes as a tool to elucidate cell wall-mediated signaling during pathogenesis* |
| *P54* | **Lucas Baiochi Riboldi** <baiochir@msu.edu> | *Optimizing CRISPR-Cas9 genome editing for precise gene deletion in Zea mays* |
| *P55* | **Sylvia Morais de Sousa Tinoco** <sylvia.sousa@embrapa.br> | *Overexpression of PSTOL1-like genes increases maize root surface area and biomass under low and high phosphorus conditions* |
| *P56* | **J.Aaron Avalos-Calleros** <aaronac089@ksu.edu> | *Phenotypic characterization of overexpression of glossy3 involved in biosynthesis of cuticular wax in maize* |
| *P57* | **Jacob Olson** <olson169@purdue.edu> | *Red chlorophyll catabolite reductase mutants in maize and sorghum* |
| *P58* | **Md Nazmul Hossain** <nhossain@iastate.edu> | *Restoration of haploid male fertility in maize: A novel approach using Zmjr mutants* |
| *P59* | **Max Braud** <mbraud@danforthcenter.org> | *Robust leaf-based transformation in Sorghum bicolor and editing of classical maize genes* |
| *P60* | **Brianna Griffin** <bdg@iastate.edu> | *Roles of REL2 mediated transcriptional co-repression in maize immunity* |
| *P61* | **Nadia Mourad** <nmourad@ufl.edu> | *Sorbitol dehydrogenase enhances kernel size by integrating carbohydrate and redox metabolism in the hypoxic endosperm* |
| *P62* | **Taylor Scroggs** <taylor.scroggs@uga.edu> | *Systematic exploration of transcription factor function in maize* |
| *P63* | **Yuguo Xiao** <yxiao@danforthcenter.org> | *Targeted seed EMS mutagenesis reveals a bHLH transcription factor underlying male sterility in sorghum* |
| *P64* | **Matthew Helm** <Matthew.Helm@usda.gov> | *The maize tar spot pathogen Phyllachora maydis encodes an effector protein that targets the chloroplasts and suppresses plant immunity* |
| *P65* | **Mae Mercado** <mercado6@illinois.edu> | *The tale of two rubisco activase: studying the function of rca1 and rca3 in maize.* |
| *P66* | **Raksha Singh** <rakshya.singh@gmail.com> | *Transcriptomic analysis of tar spot and fisheye lesions exhibit distinct yet overlapping transcriptomic signatures, suggesting a complex interplay of shared and specific pathways* |
| *P67* | **Charles Hunter** <charles.hunter@usda.gov> | *Transcriptomic and metabolomic analyses of oxylipin-deficient maize mutants* |
| *P68* | **Alessandra Koltun** <alessandra.koltun@corteva.com> | *Tropical maize: paving the way to explore untapped genetic diversity* |
| *P69* | **Holly Anderson** <hollyaa2@illinois.edu> | *Understanding anthocyanin accumulation in developing pericarp tissue through comparative transcriptomics of near-isogenic and inbred purple maize cultivars* |

*P70* **Huda Ansaf** <hah34@umsystem.edu>
*Understanding the role of TOR signaling and translational machinery in regulating protein-bound amino acid homeostasis in maize kernels*

*P71* **Jeffery Gustin** <jeff.gustin@usda.gov>
*What's new at the Maize Genetic Cooperation Stock Center*

*P72* **Wen-Yu Liu** <wliu34@ncsu.edu>
*ZmCER9-mediated regulation of autoactive NLR proteins and effector-triggered immunity via ERAD pathway*

*P73* **Tyler Ferris** <tyler.ferris@huskers.unl.edu>
*k1C resiliance: 400kb deletion of alpha kafirin family genes yields negligible nonkafirin proteome compensation in sorghum*

## Cell and Developmental Biology

*P74* **Zongliang Chen** <zc176@rutgers.edu>
*A ZmWUSCHEL1-LITTLE ZIPPER-ROLLED LEAF1/REVOLUTA regulatory module controls stem cell organization in maize inflorescences*

*P75* **Xiaosa Xu** <xjkxu@ucdavis.edu>
*A high-resolution, meristem stage-specific single-cell gene expression atlas resolving developmental dynamics in maize inflorescence architecture*

*P76* **Xiaosa Xu** <xjkxu@ucdavis.edu>
*A nuclear role of RAMOSA3 in inflorescence branching independent of its enzymatic function*

*P77* **Yaping Zhou** <yzhou2@uni-bonn.de>
*A spatiotemporal transcriptional map of maize lateral root formation*

*P78* **Harrison Bell** <bellhar@oregonstate.edu>
*Assessing factors underlying variation in maize pollen grain size: Genetics, development and environment.*

*P79* **Richie Eve Ragas** <rgr86@cornell.edu>
*Asymmetric signaling and transcription factor interactions generate robust nonrandom patterning in maize leaf margins*

*P80* **Thu Tran** <tran@cshl.edu>
*Catalytic and non-catalytic TREHALOSE-6-PHOSPHATE SYNTHASES (TPSs) interact with RAMOSA3 to control maize development*

*P81* **Kenneth Birnbaum** <ken.birnbaum@nyu.edu>
*Cell-based high throughput screening in maize*

*P82* **Denise Caldwell** <caldwed@purdue.edu>
*Cell-specific insights into fungicide mode of action and host resistance to improve tar spot management in maize*

*P83* **David Zimmerman** <zimmerman@cshl.edu>
*Characterizing vasculature reconnection in maize "Twin-Grafts"*

*P84* **Andrea Sama** <asama@ucsd.edu>
*Chemical imaging reveals metabolic responses to salt-stress in maize roots*

*P85* **Xiaosa Xu** <xjkxu@ucdavis.edu>
*Comprehensive single-cell profiling of plant shoot stem cells uncovers key insights for functional studies and trait gene discovery*

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| *P86* | **Taran Kermani** <kermani@udel.edu> | *Cross-species transcriptome comparison reveals candidate transcription factors for brace root development* |
| *P87* | **Hank Bass** <bass@bio.fsu.edu> | *Cytological, genomic, and comparative analysis of DNA replication in maize and sorghum* |
| *P88* | **Doris Cao** <dcao@udel.edu> | *Designing form and function into research devices* |
| *P89* | **Fernanda Ghenov** <fghenov@hawaii.edu> | *Determining flowering phenotypes and expression signatures of temperate and tropical maize grown in short and long day field environments* |
| *P90* | **Emilia Pierce** <emiliap@udel.edu> | *Differential expression of nitrogen transporters contributes to uptake differences between aerial and soil brace roots* |
| *P91* | **Liuyang Chu** <lchu@uni-bonn.de> | *Effects of phenylboronic acid on maize root development: Boron-related but rhamnogalacturonan II-independent?* |
| *P92* | **Abigail Tripka** <atripka@ucsd.edu> | *Exploring small molecule regulation of root development in Zea Mays* |
| *P93* | **Julian Somers** <jms53460@uga.edu> | *Exploring the timing of the sporophyte-to-gametophyte transition (SGT)* |
| *P94* | **Yan Zhou** <yzhou86@iastate.edu> | *Genes that regulate azimuthal canopy re-orientation exhibit sensitivity to plant density* |
| *P95* | **Johannes Scharwies** <joscha@stanford.edu> | *Genetic control of lateral root branching in response to moisture* |
| *P96* | **Lander Geadelmann** <landerg@iastate.edu> | *Genetic networks of maize phyllotaxy mutants* |
| *P97* | **Nicholas Francis** <francini@oregonstate.edu> | *Harnessing pluripotency: Identifying key regulators of plant cell regeneration* |
| *P98* | **Kevin Begcy** <kbegcy.padilla@ufl.edu> | *Heat stress at tricellular stage inhibits maize pollen dehiscence* |
| *P99* | **Maria Helena Faustinoni Bruno** <mfaustinonibruno@gmail.com> | *Influence of the temperature, light and nutritional status in infection by Agrobacterium tumefaciens in maize leaf transformation* |
| *P100* | **Makayla Drew** <makaylad@ucr.edu> | *Investigating TANGLED1-mediated division site maintenance across land plant lineages* |
| *P101* | **Rob Meister** <rmeister@pairwise.com> | *Leveraging LRR receptor gene editing for enhanced ear morphology in maize* |
| *P102* | **Ruqiang Zhang** <rz444@cornell.edu> | *Localized changes in developmental phase-transition on a recurrent morphogenetic regulatory network enables maize leaf angle variation* |
| *P103* | **Faith Kanana Mbiti** <faith.kanana@ur.de> | *MALE STERILE CONVERTED ANTHER 1 (MSCA1), Hypoxia and TGA Transcription Factors* |

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| *P104* **Maria Camila Medina Montes** <medinamm@oregonstate.edu> | *Making good connections: How transverse veins span the maize leaf vascular network* |
| *P105* **[Removed]** | *[Removed by request of the author]* |
| *P106* **Allyson Angermeier** <aangermeier@danforthcenter.org> | *New tools for visualizing phasiRNA biogenesis in maize anthers via hybridization chain reaction, immunofluorescence, and multiplex super-resolution microscopy.* |
| *P107* **Brad Nelms** <nelms@uga.edu> | *Pizza theory! Meristem organization restricts the spread of new mutations among the offspring* |
| *P108* **Lukas Evans** <Le95@cornell.edu> | *Positional cell divisions, growth, and recruitment contribute to cell-fate acquisition at a developmental boundary in the maize leaf* |
| *P109* **Vishadinie Jayasinghe** <jmvisha@iastate.edu> | *Response of autophagy-deficient maize atg10 mutants to heat stress* |
| *P110* **Thanduanlung Kamei** <thanduan@udel.edu> | *SBP mutants have an expanded competence zone for brace root initiation* |
| *P111* **Liam German** <ltg27@cam.ac.uk> | *STR2 – a plant half-sized ABCG transporter with a multifaceted role in AM symbiosis* |
| *P112* **Brian Zebosi** <zebosib@oregonstate.edu> | *Sex and death: Mapping and characterization of several novel tassel seed mutants with altered sex determination* |
| *P113* **John Hodge** <jgerardhodge@gmail.com> | *Spatial analysis of stomatal patterning can quantify component traits tied to developmental genetics of stomatal density in maize* |
| *P114* **Lily O'Connor** <loconnor@danforthcenter.org> | *Supporting the next generation: bHLH transcription factor complexes regulating maize anther development* |
| *P115* **Michael Busche** <busche.michael@gmail.com> | *TARGET OF RAPAMYCIN regulates gene expression across developmental gradients* |
| *P116* **Nandhakumar Shanmugaraj** <shanmug@cshl.edu> | *TERMINAL EAR1 (TE1) couples maize development with stress responses* |
| *P117* **Dayane Cristina Lima** <dclima@wisc.edu> | *The Wisconsin Crop Innovation Center: a public resource for crop transformation and gene editing research* |
| *P118* **Hailong Yang** <hailongyang@umass.edu> | *The cis-regulatory evolution of GRASSY TILLERS1 (GT1) in the grass family* |
| *P119* **Prameela Awale** <pa96f@umsystem.edu> | *The enhancer of spi1 (eos1) gene functions in maize tassel development by regulating auxin transport* |
| *P120* **Jiaqi Zhou** <jizhou@cshl.edu> | *Uncovering maize regeneration mechanism through morphogenic regulators mutants* |
| *P121* **Irene Ikiriko** <iikiriko@udel.edu> | *Uncovering the genes regulating lifespan stalk flexural stiffness* |
| *P122* **Emma Klaas** <eklaas43@gmail.com> | *lateral rootless2 (lrs2) shapes maize root development by regulating polar auxin transport* |

## Computational and Large-Scale Biology

*P123* **Magdalena Janik**
<j.magdalena@wustl.edu>
*A comparative approach for selecting orthologous candidate genes underlying signal in elemental accumulation genome-wide association studies across multiple species*

*P124* **Jonathan Ojeda-Rivera**
<joo29@cornell.edu>
*A large-scale transcriptomic survey of nitrogen recycling genes in perennial grasses for sustainable maize production*

*P125* **Carson Andorf**
<carson.andorf@usda.gov>
*A unified VCF data set from nearly 1,500 diverse maize accessions and resources to explore the genomic landscape of maize*

*P126* **Carson Andorf**
<carson.andorf@usda.gov>
*Using the MaizeGDB genome browser to evaluate gene model annotation quality and gain functional insights*

*P127* **Ethalinda Cannon**
<ethy.cannon@usda.gov>
*Updating the maize nomenclature recommendations*

*P128* **Jack Gardiner**
<jack.m.gardiner@gmail.com>
*Maizemine: Tools for mining gene expression in the maize NAM lines*

*P129* **Olivia Haley**
<olivia.haley@usda.gov>
*Comparing the performance of protein folding models AlphaFold, ESMFold, and Boltz for classical genes in maize*

*P130* **Olivia Haley**
<olivia.haley@usda.gov>
*Fusarium protein toolkit: An AI-based resource to facilitate exploring host-pathogen interactions in maize*

*P131* **Laura Tibbs-Cortes**
<laura.tibbs-cortes@usda.gov>
*Plasticity and fitness trade-offs in switchgrass revealed by open science and citizen science data*

*P132* **John Fowler**
<fowlerjo@oregonstate.edu>
*Alleles that reduce pollen fitness can condition heterogeneous spatial patterns of inheritance across maize ears*

*P133* **Huan Chen**
<chenhua9@msu.edu>
*Archaeological Bolivian maize genomes suggest Inca cultural expansion augmented maize diversity in South America*

*P134* **Wei-Yun Lai**
<wl748@cornell.edu>
*Building a practical haplotype graph from PacBio assemblies for genotype imputation and allele mining in maize*

*P135* **Yue Liu**
<yueliu@iastate.edu>
*Candidate genes underlying a major QTL qshgd1 causing spontaneous haploid genome doubling in maize A427*

*P136* **Sontosh Deb**
<skdeb@ncsu.edu>
*Characterization of allele-specific expression and cis-regulatory divergence in hybrid maize: Implications for gene-environment interactions*

*P137* **Jingjing Zhai**
<jz963@cornell.edu>
*Cross-species modeling of plant genomes at single nucleotide resolution using a pre-trained DNA language model*

*P138* **Yan Zhou**
<yzhou86@iastate.edu>
*Dancing canopy: Genes regulate azimuthal canopy orientations and response to high planting density in maize*

*P139* **Szu-Ping Chen**
<sc3367@cornell.edu>
*Decoding tissue-specific gene expression using DNA language model*

*P140* **Jaspreet Sandhu**
<jsandhu@danforthcenter.org>
*Dissecting the physiological and molecular responses to nitrogen stress in sorghum*

*P141* **Alejandra Quinones**
<quino070@umn.edu>
*Dynamic patterns of maize phenotypic responses to drought, heat, and their combined stress*

*P142* **Micah Kelleher**
<mkelleher@danforthcenter.org>
*Expanding maize breeding resources with teosinte alleles through a practical haplotype graph map of the Zea Synthetic population*

*P143* **Riley Harper**
<riley.harper@ufl.edu>
*Exploring the influence of nitrogen on rhizosphere and apoplastic microbiomes in maize*

*P144* **Mohamed El-Walid**
<mze3@cornell.edu>
*Genomic assembly and analysis of fast-flowering mini-maize*

*P145* **Zhenyuan Lu**
<luj@cshl.edu>
*Gramene plants: Empowering agricultural research from genomic diversity through pan-genome analysis*

*P146* **Jacob Washburn**
<jacob.washburn@usda.gov>
*High-throughput phenotyping with high resolution maize ear and kernel imaging*

*P147* **Jensina Davis**
<jdavis132@huskers.unl.edu>
*How much of phenotypic variation across environments is explained by variation in gene expression?*

*P148* **Rommel Jr. Garrido**
<garri318@umn.edu>
*Impacts of structural variant deletions and InDels on SNP genotype imputation*

*P149* **Daniel Kick**
<daniel.kick@usda.gov>
*Incorporation of biological information in deep learning models for phenotypic prediction*

*P150* **Marie-Laure Martin**
<marie-laure.martin@inrae.fr>
*Integration of phenomic, proteomic, and genomic data into a multi-scale network unravels missing heritability for maize response to water deficit*

*P151* **Sofiya Arora**
<sarora3@unl.edu>
*Leveraging natural genetic variation for functional validation of genes with predicted functions in maize*

*P152* **Rohit Kumar**
<mohank@clemson.edu>
*Localized and systemic nature of metabolic responses in root-mycorrhizae interactions*

*P153* **Katherine Murphy**
<kmurphy@danforthcenter.org>
*Low-cost, image-based phenotyping of maize abiotic stress responses*

*P154* **Doreen Ware**
<ware@cshl.edu>
*Maize data for biology, breeding, and genomics: The PanMaize Gramene database*

*P155* **Worasit Sangjan**
<worasitsangjan.ws@gmail.com>
*MatchPlant: An open-source pipeline for UAV-based single-plant detection and data extraction*

*P156* **Rijan Dhakal**
<rdhakal@danforthcenter.org>
*PanvaR: An ergonomic candidate gene discovery workflow in R.*

*P157* **Aditya Raj**
<adiraj@iastate.edu>
*Phenobot - A robotic platform for field-based corn plant phenotyping via multi-sensor fusion*

*P158* **Sebastian Mueller**
<Muellese@oregonstate.edu>
*Predictive modeling of pollen fitness phenotypes from genome scale data identifies expression specificity as a critically informative parameter*

*P159* **Meera Rajagopal**
<rajagopal.54@buckeyemail.osu.edu>
*Quantifying transposable element expressions: Utilizing computational biology and comprehensive annotations to recognize various features in RNA-seq data*

*P160* **Zong-Yan Liu**
<zl843@cornell.edu>
*ReelGene2: A large language model for single base pair precision gene annotation in diverse plant genomes*

*P161* **Sunil Kenchanmane Raju**
<kenchanmane@gmail.com>
*Role of chromatin accessibility in adaptive divergence of duplicate paralogs*

*P162* **Nikee Shrestha**
<nshrestha5@huskers.unl.edu>
*Searching for conserved alternate splicing regulation in maize and sorghum*

*P163* **Ryleigh Grove**
<rgrove4@huskers.unl.edu>
*Sweet Angle O' Mine: A comparative analysis of leaf angle phenotyping in sorghum*

*P164* **Zhongjie Ji**
<zji7@unl.edu>
*Temporal UAV surveys for robust and accurate maize flowering time prediction*

*P165* **Zachary Miller**
<zrm22@cornell.edu>
*The practical haplotype graph version 2: a streamlined and simple pangenome system*

*P166* **Quinn Johnson**
<qvj2@cornell.edu>
*Tracking the genomic location and chromatin context of meiotic double-strand breaks leading to crossovers*

*P167* **Alexandria Tran**
<tran30@illinois.edu>
*Transcriptomic analysis of maize heterosis by nitrogen use efficiency*

*P168* **Colin Finnegan**
<cpf@iastate.edu>
*UTR development in maize*

*P169* **Anuradha Singh**
<singha57@msu.edu>
*Unlocking sorghum's growth and yield potential: a study on nitrogen regimes and biostimulant applications*

*P170* **Henry Dawson**
<hdd29@cornell.edu>
*Unveiling cold tolerance in maize: Linking chloroplast genomics and environmental adaptation for climate-resilient breeding*

*P171* **Cole Hammett**
<chhammet@ncsu.edu>
*Using UAV imagery to assess foliar disease resistance*

*P172* **John Fowler**
<fowlerjo@oregonstate.edu>
*Using the Ds-GFP population to assess the contributions of pollen-expressed genes to maize reproduction*

*P173* **Morgan Mathison**
<mgmb7w@umsystem.edu>
*Utilizing drone imaging in analysis of genotype by environment in maize*

*P174* **Edward Cedrick Fernandez**
<edward.fernandez@ndsu.edu>
*Within-leaf spatial responses to heat stress reveal varied genetic mechanisms in Hordeum vulgare*

*P175* **Christopher Topp**
<ctopp@danforthcenter.org>
*X-ray tomography provides nondestructive multiscale 3D imaging to support maize research*

## Cytogenetics

*P176* **Taylor Isles** <ti97d@umsystem.edu>
*Analysis of McClintock's tiny fragment/X component*

*P177* **Paul Chomet** <pchomet@gmail.com>
*Capturing snapshots in history: Images from a collection of B. McClintock's microscope slides*

*P178* **Wojtek Pawlowski** <wp45@cornell.edu>
*Chromosome axis provides scaffold for splicing meiotic gene transcripts*

*P179* **Hua Yang** <yanghu@missouri.edu>
*Copy number variation analysis of the cis region required for the B chromosome non-disjunction in maize*

*P180* **Mateus Mondin** <mmondin@usp.br>
*Cytogenetic and genetic insights into non-mendelian segregation, non-disjunction, and phenotypic expression in the maize A-B chromosomal translocation line TB-9Sb*

*P181* **Mateus Mondin** <mmondin@usp.br>
*Effects of B chromosomes on flowering time in Zapalote Chico inbred lines and the Cateto x Zapalote Chico hybrid*

*P182* **Malika Sharma** <msb92@umsystem.edu>
*Genome-Wide investigation of recombination in the presence of B chromosomes*

*P183* **Nathan Gilbert** <negvgc@umsystem.edu>
*Multiple trans-acting factors are required for B chromosome nondisjunction*

*P184* **Kelly Dawe** <kdawe@uga.edu>
*Reshaping the maize karyotype using synthetic centromeres*

*P185* **Hua Yang** <yanghu@missouri.edu>
*Single gene example of genomic balance using transcription factor B-peru on the B chromosome*

*P186* **Sora Haagensen** <sorahaagensen@brandeis.edu>
*Understanding the role of PRD2 in regulating meiotic recombination in maize*

*P187* **Kempton Bryan** <kempton@uga.edu>
*Utilization of engineered neochromosomes to study breakage-fusion-bridge cycle outcomes*

## Education & Outreach

*P188* **Vivian Bernau** <vivian.bernau@usda.gov>
*Managing and distributing maize diversity: The NCRPIS maize collection*

*P189* **Jaclyn Nicole Uy** <uyjnr69@hawaii.edu>
*Reinventing tissue culture and transformation in the classroom: TPSS 499 - Track 2*

*P190* **Katy Guthrie** <guthr103@umn.edu>
*Teaching scientific writing alongside the scientific method in an introductory plant biology lab.*

*P191* **Brandi Sigmon** <bsigmon2@unl.edu>
*The MaGNET program: Fostering community and building networks through effective mentorship for early career scientists*

*P192* **Paul Scott** <paul.scott@usda.gov>
*The organic corn breeding boot camp: An outreach activity for students, farmers and scientists*

## Quantitative Genetics & Breeding

*P193* **Alexander Liu**
<aliu@danforthcenter.org>
*A Rootless1 knockdown allele affects maize nodal root development, increasing rooting depth, nitrogen uptake efficiency, and grain production in the field*

*P194* **Matthew Runyon**
<mrunyon2@illinois.edu>
*A job for a cob? Enhancing cob sink strength as an approach for programming staygreen in maize*

*P195* **Christopher Topp**
<ctopp@danforthcenter.org>
*A longitudinal study of the impacts of cover crop diversity on maize yield and nitrogen uptake*

*P196* **Tao Zhong**
<tzhong3@ncsu.edu>
*A mitogen-activated protein kinase kinase kinase gene conferring quantitative foliar disease resistance in maize*

*P197* **Edoardo Bertolini**
<EBertolini@danforthcenter.org>
*A network-informed genomic prediction approach for plant architecture traits in maize and sorghum*

*P198* **Melissa Draves**
<madcfr@umsystem.edu>
*Phenotypic evaluation of U.S. maize heirlooms in Columbia, MO*

*P199* **Jordan Cummings**
<jcummin5@ncsu.edu>
*Phenotypic evaluation and genotypic characterization of U.S. maize heirlooms in Clayton, NC*

*P200* **August Thies**
<athies@danforthcenter.org>
*Analyzing the impact of breeding, planting density and nitrogen availability on maize root system architecture using X-ray imaging*

*P201* **Brandon Webster**
<webst250@msu.edu>
*As above leaf below: integrating drone imagery and leaf gas exchange measurements from the Wisconsin Diversity Panel in different Nitrogen treatments*

*P202* **Hannah Pil**
<hdpil@ncsu.edu>
*BZea: A diverse teosinte introgression population for improving modern maize sustainability*

*P203* **Jonathan Niyorukundo**
<niyorukundoj@gmail.com>
*Breeding for high lysine content and color diversity in popcorn and sweet corn to enhance their macro- and micronutrient profiles*

*P204* **Anthony Assibi Mahama**
<maassibi@gmail.com>
*Breeding for reduced maize grain nitrogen content*

*P205* **Samuel Herr**
<skh77@cornell.edu>
*Breeding maize stover for a circular bioeconomy*

*P206* **Cinta Romay**
<mcr72@cornell.edu>
*CERCA - Circular Economy that Reimagines Corn Agriculture*

*P207* **Michael Burns**
<burns756@umn.edu>
*CHIP-NMC: an application for corn hybrid and inbred prediction of nixtamalization moisture content*

*P208* **Alexander Mullens**
<mullens3@illinois.edu>
*Characterizing tissue-specific disease resistance to Xanthomonas vasicola pv. vasculorum in maize*

*P209* **Nate Korth**
<njkorth@ncsu.edu>
*Characterizing untapped genetic diversity in maize and its relatives to regulate the rhizosphere microbiome and improve crop resilience*

*P210* **Amanpreet Kaur**
<kaur60@purdue.edu>
*Cis-acting natural variation in transcript abundance affects the phenotype of the semi-dominant D13-1 mutant*

*P211* **Nathan Bowser**
<nbowser@iastate.edu>
*Combining ability and heterotic responses among newly developed elite stay-green sorghum inbred lines*

*P212* **Xianran Li**
<xianran.li@usda.gov>
*Complex traits: Genetic diversity, environmental context, and phenotypic plasticity*

*P213* **Kiara Kappelmann**
<kiarak@iastate.edu>
*Comprehensive kernel analysis in large DH populations derived from intercrossing germplasm enhancement of maize lines*

*P214* **Greg Ziegler**
<gziegler@gmail.com>
*Construction of a high-density, sequence-indexed Setaria viridis NMU mutant population*

*P215* **Ashley Hostetler**
<ahende@udel.edu>
*Decoupling brace root development from phase change in sorghum: candidate genes revealed by GWAS*

*P216* **Karlene Negus**
<knegus@iastate.edu>
*Designing AI models for genomic selection*

*P217* **Lara Brindisi**
<lara.brindisi@usda.gov>
*Dissecting the genetic architecture of cold acclimation and freezing tolerance in Tripsacum using eQTL mapping*

*P218* **Xiquan Gao**
<xgao@njau.edu.cn>
*Dissection of genetic basis of maize resistance to Gibberella Ear Rot and its associated seed microbiome*

*P219* **Nina Chumak**
<nina.chumak@botinst.uzh.ch>
*Engineering synthetic apomixis in maize*

*P220* **Matthew Wendt**
<wendtmat@iastate.edu>
*Environmental and genetic factors underlying maize cuticular wax accumulation under drought stress*

*P221* **Joseph Romano**
<jromano@udel.edu>
*Evaluating early vigor in maize genotypes under pythium root rot stress: A pilot experiment*

*P222* **Beatrice Konadu**
<bak234@cornell.edu>
*Exploring trade-offs between drought and cold tolerance in Tripsacum dactyloides.*

*P223* **Steve Moose**
<smoose@illinois.edu>
*Expression variation in Glossy15 is associated with maize harvest index.*

*P224* **Xuan Liu**
<xuanliu@iastate.edu>
*Field-scale and high-throughput maize leaf angle characterization using stereo vision and deep learning*

*P225* **Caner Yavuz**
<cyavuz2@unl.edu>
*Fine mapping of QTLs for candidate genes responsible for embryogenic callus induction in maize*

*P226* **Donielle Brottlund**
<brottlu1@msu.edu>
*From field to future: Environmental impacts on parental and progeny corn traits*

*P227* **Luke Gregory**
<lmg342@cornell.edu>
*From point clouds to canopy architecture insights: High-throughput trait extraction from UGV-based LIDAR in maize*

*P228* **Dikshya Sapkota**
<dikshya2@illinois.edu>
*Genetic analyses of novel inflorescence architecture traits in broomcorn*

*P229* **John Searl**
<jsearl@wisc.edu>
*Genetic analysis of fertility restoration in cytoplasmic male sterility-C type using WI-SS-MAGIC population and ex-PVP maize lines*

*P230* **Shalma Maman**
<Shalma.Maman@jacks.sdstate.edu>
*Genetic architecture underlying mean and plasticity of kernel traits in maize highlights independent control and key loci*

*P231* **Waqar Ali**
<wali3@huskers.unl.edu>
*Genetic dissection and characterization of yield component traits in maize*

*P232* **Pearl Abu**
<plabu@wacci.ug.edu.gh>
*Genetic diversity and inter-trait relationship of tropical extra-early maturing quality proteinmaize inbred lines under low soil nitrogen stress*

*P233* **Jake Hinrichsen**
<jhinrich@iastate.edu>
*Genome-wide dissection of leaf angle variation across the canopy in maize*

*P234* **Yu-Ru Chen**
<yuruchen@iastate.edu>
*Genomic prediction insights across haploid and diploid levels in maize*

*P235* **Jianming Yu**
<jmyu@iastate.edu>
*Genomic selection: Essence, applications, and prospects*

*P236* **Lizeth Dominguez**
<lizethd2@illinois.edu>
*Getting priorities straight: Altering source-sink strength in Sorghum bicolor increases sugar production*

*P237* **Huyu Liu**
<huyu@iastate.edu>
*High intensity phenotyping sites: Genetic regulation of phenotypic plasticity/stability.*

*P238* **Vitor Sagae**
<vitor.sagae@ufl.edu>
*Hybrid prediction in CHiDO: A friendly no-code genomic prediction tool for breeders*

*P239* **Vencke Gruening**
<venckegr@iastate.edu>
*Impact of spontaneous haploid genome doubling on haploid induction in maize haploid inducers*

*P240* **Qin Yang**
<qyang@nwafu.edu.cn>
*Inactivation of a lysine-histidine transporter-1 gene confers southern leaf blight resistance in maize*

*P241* **Erin Farmer**
<eef52@cornell.edu>
*Integrating proximal sensing modalities for enhanced prediction of agronomically important crop traits*

*P242* **Tae-Chun Park**
<tcpark@iastate.edu>
*Integration of doubled haploid technology with marker-assisted techniques for fixing major genes to develop specialty corn*

*P243* **Shuya Wang**
<shuyaw2@illinois.edu>
*Investigating the role of a group XIIa LRR receptor-like kinase in maize resistance against Xanthomonas vasicola pv. vasculorum*

*P244* **Manoj Subedi**
<msubedi@udel.edu>
*Leveraging UAV-based hyperspectral imaging and machine learning models for prediction of SPAD in maize*

*P245* **Ally Schumacher**
<schum193@msu.edu>
*Maize genomic prediction: Integrating genomics and transcriptomics for trait analysis*

*P246* **Sarah Lipps**
<slipps@illinois.edu>
*Maize hybrid stability and weather variables associated with ear rot resistance and mycotoxin accumulation*

*P247* **Greg Schoenbaum**
<gregorys@iastate.edu>
*Managing plot orientation and canopy architecture to improve overall maize grain yield*

*P248* **Olamide Adesina**
<oadesina@ksu.edu>
*Mining genetic resistance to Goss's wilt of maize*

*P249* **Ty Thomas**
<tsthoma2@ncsu.edu>
*Multi-environmental transcriptome-wide association study reveals the extent of genotype-by-environment interactions for flowering time in maize.*

*P250* **Vladimri Torres-Rodriguez** <jtorres-rodriguez2@unl.edu>
*Multi-species transcriptome-wide association studies identify additional genes controlling flowering*

*P251* **Prashant Bhandari** <prash.danforth@gmail.com>
*Multiple conserved loci underlie plant water use efficiency in Sorghum and Setaria*

*P252* **Lukas Würstl** <l.wuerstl@tum.de>
*Natural alleles of the gene lhcb6 shape photosynthesis and key agronomic traits in maize (Zea mays L.) landraces*

*P253* **Aaron Kusmec** <amkusmec@ksu.edu>
*Natural and orthogonal interaction models for gene-environment and gene-gene interactions*

*P254* **Catherine Li** <chli6@illinois.edu>
*Non-destructive visualization of alpha-zein expression and grain protein in maize using the FLOURY2-RFP reporter transgene*

*P255* **James Holland** <Jim.Holland@usda.gov>
*Oaxacan green dent maize is not from Oaxaca*

*P256* **Shreejana KC** <sreezaa20@gmail.com>
*Optimizing maize growth in growth chambers*

*P257* **Zhaocheng Xiang** <zxiang2@unl.edu>
*Parametrization and quantification of maize leaf morphology for phenotyping and quantitative genetics*

*P258* **Gen Xu** <gxu6@unl.edu>
*Patterns of selection for adaptation to spatial and temporal fluctuating nitrogen availability in maize*

*P259* **Kirsten Hein** <khein@danforthcenter.org>
*Phenome-to-genome insights for evaluating root system architecture in field studies of maize*

*P260* **Jeonghwa Kim** <jhkim@udel.edu>
*Phenotypic and photosynthetic responses of maize germplasm to waterlogging stress*

*P261* **Dongdong Li** <ddli@iastate.edu>
*Phenotypic plasticity of flowering time is associated with cis-regulatory elements variations*

*P262* **Denise E. Costich** <dc58@cornell.edu>
*Phenotypic selection of maize adapted to temperature extremes as a strategy to maintain productivity under global climate change, part 2*

*P263* **Harshita Mangal** <hmangal2@huskers.unl.edu>
*Population-level study on nitrogen stress responses in sorghum*

*P264* **Collin Luebbert** <cluebbert@danforthcenter.org>
*Pre-breeding maize for enhanced performance in no-till cover cropping systems using a teosinte synthetic population*

*P265* **Vinay Chaudhari** <vchaudhari@danforthcenter.org>
*Predicting end-of-season Sorghum biomass from seedling-stage traits*

*P266* **Libia F. Gomez-Trejo** <lgomez5@unl.edu>
*Rust nonhost resistance responses in sorghum and maize*

*P267* **Forrest Li** <frrli@ucdavis.edu>
*Sequencing a seed bank: Assessing the utility of environmental data from CIMMYT traditional varieties for climate-adaptive maize breeding*

*P268* **Jacob Kelly** <jakhdd@missouri.edu>
*Speed breeding fast-flowering mini-maize*

*P269* **Juliana Yassitepe**
<juliana.yassitepe@embrapa.br>
*Temporal field-based phenomics for evaluating transgenic maize under drought stress*

*P270* **Qiuyue Chen**
<qchen295@wisc.edu>
*Ten years of Genomes to Fields: a collaborative corn breeding effort*

*P271* **Jiawen Lu**
<Carmen_lujw@163.com>
*Tensor decomposition reveals trans-regulated gene modules in maize drought response*

*P272* **Chad Soenksen**
<chad.soenksen@colostate.edu>
*Testing the role of copy number variation in adaptation to drought using a pangenome approach*

*P273* **Yipu Li**
<liyipu1987@163.com>
*The ZmCCT10 transcription factor promotes DNA-mediated RNA polymerase beta expression, enhancing resistance to salt stress and improving yield in maize*

*P274* **Emma Leary**
<enltz5@mail.missouri.edu>
*The cold case: Characterizing photosynthetic performance at suboptimal temperatures using diverse germplasm*

*P275* **Heather Wodehouse**
<wodehouse@wisc.edu>
*Understanding the genetic impact of divergent selection on vegetative phase change in maize*

*P276* **Yun Luo**
<yl3956@cornell.edu>
*Unlocking the secrets of ear length and frost tolerance in maize and Tripsacum*

*P277* **Qi Mu**
<qimu@udel.edu>
*Unraveling a MYB transcription factor controlling plant height and involved in phenotypic plasticity in sorghum and maize*

*P278* **Sarah Fitzsimmons (Oliver)**
<sloxmd@umsystem.edu>
*Unraveling the genetic architecture of free asparagine in maize kernels*

*P279* **Peyton Sorensen**
<peytonls@illinois.edu>
*Untangling specificity: Investigating tissue-specific responses to bacterial pathogens in maize*

*P280* **Robert Twohey III**
<twohey2@illinois.edu>
*Using a high-throughput screening method to compare chamber and field grown VPD transpirational breakpoints in Zea mays*

*P281* **Zachary Traylor**
<zbtyxb@umsystem.edu>
*Validating a lab-scale ethanol production method for exploring metabolites in craft distilling*

*P282* **Veronica Justen**
<veronica.justen@uwrf.edu>
*Variation in temporal growth patterns of inbred lines and hybrid offspring*

*P283* **Kyle Swentowsky**
<swentow@cshl.edu>
*Zea diploperennis perennial regrowth QTL regrowth1 and regrowth3 control life history traits*

*P284* **Michelle Cho**
<mcho@danforthcenter.org>
*ZmIRA1: A novel genetic factor of root system architecture in maize identified by GWAS in a long-term selection population for kernel nitrogen content*

## Transposons & Epigenetics

*P285* **Jason Lynn**
<jlynn@cshl.edu>
*AGO2 and AGO3 regulate RNAi fidelity by suppressing RNA-directed DNA methylation*

*P286* **Patrick Gardner** <gardnerp1@montclair.edu>
*Active LTR transposable element annotation via a computational pipeline - LTRAnnotator*

*P287* **Carmen Rodriguez** <rodriguez.1523@osu.edu>
*Adapting Fiber-seq for mapping chromatin accessibility in maize under cold stress*

*P288* **Jeff Chen** <zjchen@austin.utexas.edu>
*An epigenetic basis for inbreeding depression in maize*

*P289* **Rajdeep Khangura** <rkhangur@purdue.edu>
*An exapted transposase modifies the Mu-suppressible allele of a lesion-forming UROPORPHYRINOGEN III SYNTHASE mutant*

*P290* **Xuelian Du** <xuelian@uni-bonn.de>
*BonnMu – A resource for functional genomics in maize (Zea mays L.)*

*P291* **Caleb Gooden** <gooden.67@osu.edu>
*Cis-regulatory elements in maize early development derived from long terminal-repeat retrotransposons.*

*P292* **Jonathan Cahn** <cahnjonathan@gmail.com>
*Enhancers and enhancer RNAs recapitulate the domestication focus on maize ears*

*P293* **Xuelian Du** <xuelian@uni-bonn.de>
*Functional characterization of the dizzy1 (diz1) dwarf maize mutant from the BonnMu resource*

*P294* **Damon Lisch** <damon.lisch@gmail.com>
*Genetic perturbation of the Mu-accessible chromatin landscape*

*P295* **Claire Menard** <menar060@umn.edu>
*Identifying novel transposable element insertions from short-read data using SWIF-TE*

*P296* **Xingli Li** <li.14356@osu.edu>
*Investigation of TEs DNA methylation patterns through long-read sequencing*

*P297* **Sicong Wang** <swang76@pride.hofstra.edu>
*Investigation of the maintenance mechanism on the silenced Ac transposon in id4*

*P298* **Beibei Liu** <beiliu@ucdavis.edu>
*Landscape: The evolution of transposable elements (TEs) in Maize*

*P299* **Dafang Wang** <dafang.wang@hofstra.edu>
*Mechanisms of small RNA-induced epigenetic silencing of Ac transposons in maize*

*P300* **Justin Scherer** <jts34805@uga.edu>
*Somatic insertional preference of the mutator transposon across distinct maize tissues*

*P301* **Yirui Sun** <s.yirui@uga.edu>
*TE-like DNA methylation in regulation of highly expressed nutrient transfer and storage genes in endosperm*

*P302* **Mark Minow** <mam34190@uga.edu>
*Using parent-offspring pairs to study the inheritance patterns of Zea mays chromatin accessibility.*

*P303* **Mohammad Mahmood Hasan** <m.hasanbot@gmail.com>
*mop1 reshapes recombination landscapes by altering DNA methylation and chromatin states at MITEs*

# Plenary Speaker Abstracts

**Keynote Speaker 1** **Thursday, March 6 7:20 PM**

## Genes, jeans, genomes, and the wondrous cycles of polyploidy in plants

(submitted by Jonathan Wendel <jfw@iastate.edu>)
Full Author List: Wendel, Jonathan F.[1]
[1] Iowa State University, Ames, Iowa USA 50011

One of the signal realizations of the genomics era is that all flowering plants are multiply polyploid, varying in the number and relative antiquity of their episodic, whole-genome doubling events. *Gossypium*, the cotton genus, exemplifies this recurrent, episodic polyploidization, with both ancient polyploidy and more recent neoallopolyploids that originated following a biological reunion 1-2 MYA of divergent diploids from different hemispheres. This serendipitous merger between diploid genomes that vary two-fold in size generated myriad genomic and transcriptomic responses, which serve as illustrative models for understanding evolutionary processes following allopolyploidy. Genomic processes include homoeologous exchange, gene silencing, intergenomic gene conversion, and novel cytonuclear interactions. Allopolyploid formation also induces complex transcriptomic responses, including genome-wide modification of genic expression and co-expression patterns and variable *cis*- and *trans*-controls governing duplicate gene expression. Cyclical, recurring polyploidy occurring over time scales ranging from hundreds to millions of years sets in motion processes that lead to genome downsizing, genomic fractionation, and chromosomal diploidization. This polyploidy-induced dynamism, observed in *Gossypium,* is episodically and variably reiterated throughout the angiosperms. A major challenge is to connect these long and short-term processes to our understanding of the genotype-to-phenotype equation, and hence the adaptive role of polyploidy and its importance to the generation of biodiversity and to agriculture.

Funding acknowledgement: National Science Foundation (NSF)

**Keynote Speaker 2** **Friday, March 7 11:30 AM**

## Input, Insurance, Objective: Reflections on diversity from the history of crop science

(submitted by Helen Anne Curry <hacurry@gatech.edu>)
Full Author List: Curry, Helen Anne[1]
[1] Georgia Institute of Technology; Atlanta, Georgia, USA 30316

Today, diversity is an established organizational and community value—one that is increasingly also the object of political debate and division. Establishing clarity about what diversity is and why it matters seems more important now than ever. In this talk, I consider the history of diversity as understood within one community—specifically, crop genetic diversity as understood among plant scientists and allied researchers—from the early twentieth century to today. Crop diversity has been characterized variously as a critical resource for scientific research and agricultural development; a risk-mitigation strategy amid homogenization in agricultural production; and object valued for its own sake and for the sake of those communities associated with it. I suggest that this history of the science of crop diversity helps us to better appreciate the challenges and imperatives in fostering diversity that we face today, in maize genetics and beyond.

Funding acknowledgement: Wellcome Trust

**Keynote Speaker 3** **Saturday, March 8 9:00 AM**

## You need a real maize geneticist

(submitted by Ivan Baxter <ibaxter@danforthcenter.org>)
Full Author List: Baxter, Ivan[1]
[1] Donald Danforth Plant Science Center; St. Louis, Mo

Plant growth and water use are interrelated processes influenced by the genetic control of both morphological and biochemical characteristics. Improving plant water use efficiency (WUE) to sustain growth in different environments is an important breeding objective that can improve crop yields and enhance agricultural sustainability. However, using traditional genetic methods to increase WUE has proven difficult due to low throughput and environmental heterogeneity encountered in field settings. To overcome these limitations, we used a high-throughput phenotyping platform to quantify plant size and water use of populations from two Panicoid C4 species under water availability contrasts. Leveraging the tight environmental control of the system, we collected samples for extensive untargeted metabolomics to understand the biochemical response of the plants. We used genome-wide association analysis to identify loci controlling the hundreds of physiological traits and thousands of metabolite traits. Comparing across species allows us to identify loci that are conserved across Panicoid evolution. We are leveraging pan-genome and transcriptome resources to identify candidate genes for these traits to facilitate targeted improvement of WUE in crop plants.

Funding acknowledgement: Department of Energy (DOE)

## Environmental integration with root cell type development

(submitted by Siobhan Brady <sbrady@ucdavis.edu>)
Full Author List: Brady, Siobhán M.[1]
[1] Howard Hughes Medical Institute, University of California

A plant's roots serve as a major line of defense against environmental stress to protect the plant as a whole. Roots of diverse plant species have found ways to deal with stress by devising cell wall modifications and natural barriers to resist drought, flooding, mineral deficiencies, and other insults that impair plant growth. Many plant species have evolved unique cell wall forms composed of specialized biopolymeric metabolites that are deposited in elegant patterns in specific cell types. These walls are largely molecularly understudied although they are linked with a variety of stress responses. I will describe my group's approaches that merge classic developmental genetics with systems biology to elucidate these developmental programs in multiple plant species.

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

**Keynote Speaker 5** **Saturday, March 8 8:05 PM**

## Doreen's Talk title placeholder

(submitted by Doreen Ware <doreen.ware@usda.gov>)
Full Author List: ware, Doreen[1]
[1] USDA-ARS, Cold Spring Harbor Lab, Laurel Hollow, New York

Doreen's abstract placeholder

# McClintock Prize Abstract

**McClintock Prize (M1)** **Friday, March 7 8:20 PM**

## Why do we do maize genetics?

(submitted by Edward Buckler <ed.buckler@usda.gov>)
Full Author List: Buckler, Edward[1]
[1] USDA-ARS, Ithaca, NY

Each of us has a unique journey into maize genetics. For me, it began with questions: How does DNA programming work? How do we produce food? Why is maize so central to this process? These inquiries have shaped my scientific path within the maize community for over 30 years. Unlike computer programming, which may seem like starting with a blank slate (though it rarely is), biology and agriculture operate within deeply complex systems shaped by billions of interactions and a few billion years of evolution.

As a geneticist seeking to design better food systems, I collaborated with this community to understand how grass evolution, maize domestication, and modern breeding shape complex traits using germplasm, genomics, field trials, and statistical and computational tools. Using these tools, we can now clearly see many commonalities in what makes the Andropogoneae grasses successful, yet the maize lineage has distinctly followed a less common genomic path to success. By tracing when and where maize was domesticated and how it adapted globally, we see that thousands of genes played a role in transforming a subtropical grass into a crop that thrives worldwide. It is now clear that maize adapts to environments through rapid regulatory turnover and genetic interplay with genome duplication, yet it remains constrained by the genetic burden of maintaining nearly 200 million bases of functional variation.

Genetics has revealed how grasses have shaped our planet, providing insights into how domestication made maize central to global agriculture. But what can be achieved in this century? Maize is a powerhouse of carbon fixation, but how can we deploy genetics to enhance its nitrogen efficiency to match that of its perennial relatives? Through genetics, physiology, agronomy, artificial intelligence, and collaboration, we can leverage this rich history and genetic inheritance to design a corn production system that is not only more productive and resilient but also has lower input costs and a reduced environmental impact. We study maize genetics because it offers insights into fundamental biological questions while playing a crucial role in shaping the future of the global food system.

Funding acknowledgement: USDA-ARS, NSF, FFAR, DOE-ARPAe

# Short Talk Abstracts

**SESSION 1 – WELCOME / KEYNOTE / GENE REGULATION**
Chair: Sherry Flint-Garcia Thursday, March 6. 7:00 PM – 9:00 PM

**T1**

## *Vgt1* as enhancer of *ZmRap2.7* impacts flowering time and gene regulatory networks involved in jasmonate signaling in maize

(submitted by Maike Stam <m.e.stam@uva.nl>)
Full Author List: Zicola, Johan[1 2]; Weber, Blaise[3]; Tu, Xiaoyu[4]; Bader, Rechien[3]; Zisis, Dimitrios[5]; Aesaert, Stijn[6]; Salvi, Silvio[7]; Krajewski, Pawel[5]; van Lijsebettens, Mieke[6]; Li, Chuanshun[4]; Li, Yangmeihui[4]; Zhong, Silin[8]; Scholten, Stefan[2]; Turck, Franziska[1]; Stam, Maike[3]
[1] Max Planck Institute for Plant Breeding, Carl-von-Linné 10, 50829 Cologne, Germany
[2] Georg-August-Universität Göttingen, Von-Siebold-Str. 8, 37075 Göttingen, Germany
[3] University of Amsterdam, Science Park 904, 1098 XH Amsterdam, The Netherlands
[4] Shanghai Jiao Tong University, Shanghai, 200240, China
[5] Institute of Plant Genetics, Polish Academy of Science, Strzeszyńska 34, 60-479 Poznań, Poland
[6] VIB-UGent Center for Plant Systems Biology, Technologiepark 9279052 Gent, Belgium
[7] DISTAL, University of Bologna,Viale Fanin 44, 40127, Bologna, Italy
[8] School of Life Sciences, The Chinese University of Hong Kong, Hong Kong, China

The identification and characterization of *cis*-regulatory DNA sequences and how they coordinate responses to developmental and environmental cues is of paramount importance to plant biology[1]. Although thousands of candidate *cis*-regulatory modules (CRMs) have been identified in maize, few of these have been well characterized[1-3]. We are studying the function of the *Zea mays* candidate enhancer *Vegetative to generative1* (*Vgt1*), a predicted regulatory element located about 70 kb upstream of the floral repressor gene *ZmRap2.7*[4]. Consistent with a function as enhancer of *ZmRap2.7*, *Vgt1* contains an accessible chromatin region. Transgenic lines containing an inverted repeat that induces DNA methylation at *Vgt1* through RNA-directed DNA methylation (RdDM) show early flowering and accelerated growth rate during early developmental stages. DNA methylation of *Vgt1* is associated with downregulated expression of the AP2-like floral repressor *ZmRap2.7* in specific leaf tissues at early developmental stages. In line with *Vgt1* regulating *ZmRap2.7*, chromosome conformation capture data shows that *Vgt1* physically interacts with the *ZmRap2.7* TSS region. Finally, chromatin immunoprecipitation of transiently expressed *ZmRap2.7* in protoplasts indicates that the ZmRAP2.7 transcription factor binds at the promoter of several hundreds of genes, including a significant proportion of genes that are differentially expressed between lines with and without DNA methylation at *Vgt1*. Altogether, we show that *ZmRap2.7* is transcriptionally controlled by *Vgt1* and is involved in flowering time and other biological pathways, such as jasmonate signaling.

1. Schmitz, Grotewold, Stam (2022) The Plant Cell 34:718. doi.org/10.1093/plcell/koab281. 2. Oka et al. (2017) Genome Biology 18:137. doi: 10.1186/s13059-017-1273-4. 3. Ricci et al (2019) Nat Plants 5: 1237. doi: 10.1038/s41477-019-0547-0. *4.* Salvi et al. (2007) PNAS 104:11376. doi: 10.1073/pnas.0704145104.
Gene / Gene Models described: *ZmRap2.7*; Zm00001eb355240
Funding acknowledgement: European Commission Seventh Framework-People-2012-ITN Project EpiTRAITS GA-316965, National Natural Science Foundation of China (NSFC, 32370247 and 32461160289), Shanghai Agricultural Science & Technology Innovation Program (T2024)

**T2**

## Decoding a complex distal non-coding QTL at *TEOSINTE BRANCHED 1*

(submitted by Ankush Sangra <ankush.sangra@uga.edu>)
Full Author List: Sangra, Ankush[1]; Chen, Zongliang[2]; Marand, Alexandre P.[1]; Mendieta, John P.[1]; Minow, Mark A.A.[1]; Clause, Haley N.[1]; Studer, Anthony J.[3]; Gallavotti, Andrea[2]; Schmitz, Robert J.[1]
[1] Department of Genetics, University of Georgia Athens, USA
[2] Waksman Institute of Microbiology, Rutgers University, Piscataway, NJ
[3] Department of Crop Sciences, University of Illinois, Urbana, IL, USA

During domestication from teosinte, maize branching and inflorescence architecture changed drastically, mainly due to increased *TEOSINTE BRANCHED 1 (TB1)* axillary bud expression. Elevated *TB1* expression is due to domestication altering a distal control region (CR) of ~11kb. This domesticated CR has four accessible chromatin regions (ACRs), three of which are consistent with enhancer activity, as they co-occur with *TB1* expression in developing female inflorescence. The final ACR appears consistent with silencer activity, as it is always accessible, even when the *TB1* locus is repressed by H3K27me3 deposition. In contrast, most teosinte contain only three ACRs, as a *Hopscotch* transposon bifurcates one ancestral ACR in domesticated maize. Here, we functionally dissect the *TB1* CR via CRISPR-Cas9 deletion of each CR ACR, *Hopscotch*, and the entire CR. Entire CR deletion phenocopy *TB1* loss of function, confirming that the CR cumulatively enhances *TB1* expression. Deletion of the two ACRs flanking *Hopscotch* caused weak *tb1* branching phenotypes, suggesting additive enhancer action; indeed, these deletions caused reduced axillary bud *TB1* expression, but no expression change in the leaf, where *TB1* is normally repressed. Deletion of the putative silencer ACR caused no overt phenotypic change, yet preliminary results indicate that it changes the chromatin accessibility of the *TB1* promoter. Surprisingly, chromatin at *Hopscotch* is not accessible and *Hopscotch* deletion produced no phenotypic changes – this, along with the presence of this *Hopscotch* insertion in select teosinte accessions, suggest changes other than the *Hopscotch* insertion caused the domestication increase in maize apical dominance. Future exploration will characterize the molecular changes accompanying our ACR deletions in the *TB1* CR and reveal more about the genetics that underpins *TB1* expression control.

Gene / Gene Models described: *tb1*; Zm00001eb054440
Funding acknowledgement: National Science Foundation (NSF), United States Department of Agriculture (USDA)

**T3**

## Cross-species modeling of plant genomes at single nucleotide resolution using a pre-trained DNA language model

(submitted by Jingjing Zhai <jz963@cornell.edu>)
Full Author List: Zhai, Jingjing[1]; Gokaslan, Aaron[2]; Schiff, Yair[2]; Berthel, Ana[1]; Liu, Zong-Yan[3]; Lai, Wei-Yun[1]; Miller, Zachary[1]; Scheben, Armin[5]; Stitzer, Michelle[1]; Romay, Cinta[1]; Buckler, Edward[1 3 4]; Kuleshov, Volodymyr[2]
[1] Institute for Genomic Diversity, Cornell University, Ithaca, NY USA 14853
[2] Department of Computer Science, Cornell University, Ithaca, NY, USA 14853
[3] Section of Plant Breeding and Genetics, Cornell University, Ithaca, NY USA 14853
[4] USDA-ARS; Ithaca, NY, USA 14853
[5] Simons Center for Quantitative Biology, Cold Spring Harbor Laboratory, 1 Bungtown Road, Cold Spring Harbor, NY USA 11724

Interpreting function and fitness effects in diverse plant genomes requires transferable machine learning models. Language models (LMs) pre-trained on large-scale unlabeled biological sequences can learn evolutionary conservation. With fine-tuning on limited labeled data, these models offer superior cross-species prediction compared to supervised approaches. Here, we introduce PlantCaduceus, a DNA LM built on the Caduceus and Mamba architectures, pre-trained on 16 angiosperm genomes spanning 160 million years of divergence. Using a masked language modeling framework, where each nucleotide is treated as a "word," PlantCaduceus captures evolutionarily constrained sequence patterns and achieves robust predictive performance with minimal labeled data. When fine-tuned on Arabidopsis for four key annotation tasks, PlantCaduceus demonstrates high transferability across divergent plant species. These tasks include translation initiation and termination site (TIS/TTS) prediction and splice donor/acceptor site identification, where it outperforms existing DNA LMs by up to 7.23-fold. For example, in maize TIS prediction, it achieves a PRAUC of 0.815, far exceeding the best baseline model's 0.089. In identifying deleterious mutations, PlantCaduceus matches the performance of state-of-the-art protein LMs and surpasses phylogenetic approaches (PhyloP/phastCons) by up to threefold without fine-tuning. Additionally, it successfully identifies known causal variants in both Arabidopsis and maize. Together, these findings highlight PlantCaduceus as a versatile and powerful foundation model with broad utility for plant genomic annotation and crop improvement.

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

**T4**

## Quantitative genetics of leaf vascular density in maize

(submitted by Diana Ruggiero <ruggiedi@oregonstate.edu>)
Full Author List: Ruggiero, Diana[1]; Chuck, George[2]; Leiboff, Samuel[1]
[1] Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331, USA.
[2] Department of Plant and Microbial Biology, UC Berkeley, Berkeley, CA 94720, USA.

$C_4$ photosynthesis requires high vascular density to shuttle carbon from mesophyll to bundle sheath cells in adjacent veins. Maize leaves achieve high vascular density by producing several vein subtypes (lateral, intermediate, small, and transverse), defined by their sequence of development and spatial configuration. To identify genes influencing vascular density across vein subtypes, we phenotyped the Wisconsin Diversity Panel (WiDiv) and conducted a genome-wide association study (GWAS). Over three field seasons, we collected 6000+ leaf samples from 760 WiDiv inbred lines and built a deep-learning phenotyping system that estimates subtype-specific vein density in images of cleared leaf tissues. The system uses two U-Net convolutional neural networks (CNN) for semantic, pixel-by-pixel image segmentation: one model performs vein subtype classification, while the second model segments leaf images into sheath, auricle, and blade, allowing for compartment specific phenotyping. We used activation mapping and 'DeepDream'-style feature visualization to show how the models interpret the leaf images. These models determined that veins make up, on average, 61% of the photosynthetically active blade and 51% of the non-photosynthetic sheath. Using publicly available field data, we found that several of our vascular phenotypes have modest, significant correlations with agriculturally relevant traits, including meristem size and flowering time. We additionally uncovered 'bundle sheath fusions' across many lines in the WiDiv, where ectopic bundle sheath cells develop in-between vascular bundles instead of mesophyll cells, violating the expected cellular spacing rules of $C_4$ Kranz anatomy. Using our GWAS pipeline, we identified 160 significant loci associated with quantitative variation from our compartment and subtype-specific vascular phenotypes. To evaluate gene candidates, we are using hybridization chain reaction (HCR) RNA *in situ* to localize transcript expression during leaf development. Preliminary physiological data shows correlations between vascular traits and stomatal conductance. Future work will determine the physiological consequences and developmental origins of these traits.

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

**T5**

# Integrating proximal sensing modalities for enhanced prediction of agronomically important crop traits

(submitted by Erin Farmer <eef52@cornell.edu>)

Full Author List: Farmer, Erin E.[1]; Michael, Peter[2]; Gu, Zeqi[5]; Yan, Ruyu[2]; Wickes-Do, Liam[1]; Lepak, Nick[3]; Romay, Cinta[4]; Buckler, Edward S.[1 3 4]; Sun, Ying[6]; Robbins, Kelly[1]; Gage, Joseph L.[7]; Davis, Abe[2]; Gore, Michael A.[1]

[1] Plant Breeding and Genetics Section, School of Integrative Plant Science, Cornell University, Ithaca, New York, USA 14853

[2] Department of Computer Science, Cornell University, Ithaca, New York, USA 14853

[3] United States Department of Agriculture-Agricultural Research Service, Robert W. Holley Center for Agriculture and Health, Ithaca, New York, USA 14853

[4] Institute for Genomic Diversity, Cornell University, Ithaca, New York, USA 14853

[5] Cornell Tech, New York, New York, USA 10044

[6] Soil and Crop Sciences Section, School of Integrative Plant Science, Cornell University, Ithaca, NY, USA 14853

[7] Department of Crop and Soil Sciences, North Carolina State University, Raleigh, North Carolina, USA 27695

Proximal sensing technologies have enabled the collection of vast amounts of phenomic data for characterization of crop traits. However, data streams collected across sensors and platforms are predominately utilized separately due to heterogeneity in their structural, spatial, and spectral information. We aim to leverage advances in artificial intelligence to characterize plant form and health across temporal scales through integration of high-dimensional, multi-modal data. We collected over 75,000 multispectral images (MSIs) via unoccupied aerial vehicles (UAVs) and over 35,000 LiDAR scans via unoccupied ground vehicles (UGVs) for maize hybrids from the Genomes to Fields project in Aurora, NY from 2020 through 2024. Autoencoders, which are unsupervised, deep learning models, were implemented to extract latent features from each data type, producing reduced representations which contained biologically relevant information, with heritabilities up to ~0.9. MSI and LiDAR latent features were integrated at the plot-level across all time points, and Bayesian regression was used to predict manually measured phenotypes. These predictions were compared against 49 vegetation indices (VIs) which had accuracies ranging from ~0.26 for stalk lodging to ~0.85 for days to anthesis. Integrated latent features outperformed VIs for all phenotypes except for flowering time traits and grain moisture, ranging from a decrease in accuracy of ~8.8% for grain moisture to an increase of ~19.0% for ear height. Across all traits, integrated latent features increased the prediction accuracy by an average of ~4.6%. Notably, integrated latent features also provided a ~5.1% and ~20.8% increase in accuracy over the individual use of MSI and LiDAR latent features, respectively. We show that latent phenotyping circumvents manual curation of features and allows integration across modalities, improving characterization of key crop traits and further facilitating the deployment of proximal sensors for use in precision agriculture and breeding programs.

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

T6

## Crowdsourcing phenotype prediction: Results from the 2024 G2F prediction competition.

(submitted by Jacob Washburn <jacob.washburn@usda.gov>)
Full Author List: Winner, Competition[1]; Washburn, Jacob D.[2]; Chen, Qiuyue[3]; de Leon, Natalia[3]; Gage, Joseph[4 5]; Holland, James[6]; Lima, Dayane[3]; Murray, Seth[7]; Romay, Cinta[8]; Taylor, Kerry[9]; Xavier, Alencar[10 11]
[1] Competition Winner Affiliation
[2] USDA-ARS, Plant Genetics Research Unit, University of Missouri, Columbia, MO 65211
[3] Department of Plant and Agroecosystem Sciences, University of Wisconsin, Madison, WI 53706
[4] Department of Crop and Soil Sciences, North Carolina State University, Raleigh, NC 27695
[5] NC Plant Sciences Initiative, North Carolina State University, Raleigh, NC, 27606
[6] USDA-ARS, Plant Science Research Unit, North Carolina State University, Raleigh, NC 27606
[7] Department of Soil & Crop Sciences, Texas A&M University, College Station, TX 77843
[8] Institute for Genomic Diversity, Cornell University, Ithaca, NY 14850
[9] Iowa Corn Promotion Board, Johnston, IA 50131
[10] Corteva Agrisciences, 8305 NW 62nd Ave, Johnston, IA, 50131, USA
[11] Department of Agronomy, Purdue University, 915 Mitch Daniels Blvd, West Lafayette, IN, 47907, USA

Predicting yield in new environments and new genetics is notoriously difficult, but accurate predictions have important ramifications for breeding, management, sustainability, and the environment. The maize Genomes to Fields (G2F) genotype by environment (GxE) project was conceived more than a decade ago as a multi-state cooperative to test hypotheses and enhance prediction of maize yield, adaptation, and environmental responses. To date the project has phenotyped around 190,000 unique plots, around 6,100 hybrids, and more than 300 environments. This year the GxE project held its second international yield prediction competition using the complete G2F GxE project dataset to date! The goal was for teams to predict the entirely unseen data collected in summer 2024. The competition attracted over 370 registrants from across the world with competitors from academia, government, non-profit, for profit, and private individuals. This presentation will announce the winning competitors to the community after which they will present the strategies, and finer details that enabled their model to outperform all others.

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

**T7**

## Ensuring the future of maize: A call for collaborative action

(submitted by Vivian Bernau <vivian.bernau@usda.gov>)
Full Author List: Bernau, Vivian[1]; Millard, Mark[1]; Mahan, Adam[1]
[1] USDA/ARS Plant Introduction Research Unit and Iowa State University College of Agriculture and Life Sciences, Ames, Iowa

The USDA National Plant Germplasm System includes a collection of more than 20,000 accessions of cultivated temperate- and tropical-adapted maize and wild relatives from around the world. This collection is distributed from the North Central Regional Plant Introduction Station (NCRPIS) in Ames, Iowa, where it is conserved at 4°C, and backed-up at the National Laboratory for Genetic Resources Preservation in Fort Collins, Colorado. The maize collection continues to grow each year, with the addition of materials with expiring plant variety protection and other resources important to the maize community. Additionally, the collection continues to age; more than half of the collection's distributable seed lots are now more than 30 years old. Funding levels for maintenance and distribution have lagged behind the demand and replenishment needs of the maize collection for some time. In order to continue conserving diversity for our cultural heritage and to address current and future challenges, we seek 1) partnerships to regenerate and characterize the maize germplasm collection and 2) feedback to aid in the prioritization of our efforts.

Funding acknowledgement: United States Department of Agriculture (USDA)

**T8**

## Translational and proteomic analysis of cold-stressed maize reveals ribosomal protein families involved in cold response and tolerance

(submitted by Veronica C Perez <vcp8@cornell.edu>)
Full Author List: Perez, Veronica C[1]; Hua, Jian[1]
[1] Plant Biology Section, School of Integrative Plant Science, Cornell University, Ithaca, New York, USA

At the onset of stress, plants undergo rapid translational reprogramming to promote the translation of response genes and minimize damage, and with extended stress periods may alter ribosomal composition to maximize response gene expression and restore overall translational efficiency and growth. In maize, exploration of this translational and ribosomal reprogramming has been sparse, especially in response to abiotic stressors such as cold. To identify genes including ribosomal protein (RP) paralogs affected at the translational level in response to cold, RNA-Seq, Ribo-Seq and MS/MS analyses were conducted on seedlings following 24hr cold stress. RNA analyses identified differentially expressed genes within total and ribosome-bound, translationally active RNA, showing both downregulation of translation and ribosome biogenesis related terms in actively translated RNA and upregulation of specific RP paralogs at the translational level. MS/MS analyses of ribosome-enriched samples identified RP families and individual paralogs that exhibited differential protein accumulation or phosphorylation in response to cold. Characterization of several cold tolerance candidate RP genes was conducted in Arabidopsis with closest homologs to determine the cold response function of these RPs. Preliminary data shows that knocking out of paralogs belonging to the uL18 and uL10 families, which showed cold responsive phosphorylation and translational induction in maize respectively, led to altered freezing tolerance and cold growth phenotypes in Arabidopsis. Together, these analyses identified several candidate RP genes that may play a role in maize or plant cold tolerance and provides a dataset for identification of other translationally regulated and cold responsive genes.

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

T9 @minorallele.bsky.social

**Introgression of a Mexican highland chromosomal inversion into temperate maize accelerates flowering, promotes growth, and modulates a cell proliferation gene network.**

(submitted by Fausto Rodríguez-Zapata <frodrig4@ncsu.edu>)
Full Author List: Rodríguez-Zapata, Fausto[1 2]; Tandukar, Nirwan[1 2]; Barnes, Allison[1]; Stokes, Ruthie[1]; Aragón-Raygoza, Alejandro[1]; Li, Meng[3]; Pérez-Limón, Sergio[3]; Perryman, Melanie[3]; Piñeros, Miguel A.[4]; Strable, Josh[1 2]; Runcie, Daniel[5]; Sawers, Ruairidh J.[3]; Rellán-Álvarez, Rubén[1 2]
[1] Molecular and Structural Biochemistry, North Carolina State University, Raleigh, North Carolina, USA
[2] Genetics and Genomics, North Carolina State University, Raleigh, North Carolina, USA
[3] Department of Plant Science, The Pennsylvania State University, University Park, PA, USA
[4] Robert W. Holley Center for Agriculture and Health, USDA-ARS, Ithaca, NY, USA
[5] Department of Plant Sciences, University of California, Davis, Davis, CA, USA

*Inv4m* is a chromosomal inversion prevalent in traditional maize varieties adapted to the cold and often phosphorus-deficient Mexican highlands. Field trials throughout Mexico have shown that, when grown at high elevations, plants carrying the inversion flower faster and have greater yield than plants without it. Although growth chamber experiments indicate that Inv4m regulates the expression of photosynthesis-related genes in response to cold, we have yet to know the genes responsible for the adaptive effects of *Inv4m* in the field. To identify *Inv4m*-regulated genes that underlie enhanced development in the field, we bred B73-based Near Isogenic Lines (NILs) with either the inversion or the standard karyotype. We then grew these NILs in phosphorus-sufficient and deficient soils to test whether *Inv4m* contributes to local adaptation through enhanced phosphorus stress response. We measured plant reproductive and vegetative traits, phosphorus, lipids, and gene expression in the leaves. Plants showed classical responses to phosphorus starvation, including decreased phosphorus and biomass accumulation, delayed flowering, and a switch from phospholipid to glycolipid production. Notably, *Inv4m* plants flowered earlier and grew taller regardless of phosphorus availability. While increased leaf age and phosphorus deficiency resulted in genome-wide expression changes, *Inv4m*'s effects were predominantly confined to genes within the inversion. Our analyses suggest that Inv4m introgression modulates a trans-coexpression network enriched in cell proliferation and flower development genes, which includes DNA replication fork genes (*pcna2*, *mcm5*), histone demethylases (*jmj2*, *jmj21*), and the *FT* florigen homolog *zcn26*. By cross-referencing with a list of candidates from the literature, we found other *Inv4m*-regulated genes associated with flowering time and plant height. In a complementary growth chamber experiment, *Inv4m* plants showed longer shoot apical meristems than controls, supporting its effect on organ development. These findings provide insights into Inv4m's role in highland adaptation through the coordinated expression of a developmental gene network.

Funding acknowledgement: National Science Foundation (NSF), United States Department of Agriculture (USDA), Science and Technologies for Phosphorus Sustainability (STEPS)

**T10**

## Integration of phenomic, proteomic, and genomic data into a multi-scale network unravels missing heritability for maize response to water deficit

(submitted by Marie-Laure Martin <marie-laure.martin@inrae.fr>)
Full Author List: Djabali, Yacine[1 2 4]; Rincent, Renaud[4]; Blein-Nicolas, Mélisande[4]; Martin, Marie-Laure[1 2 3]

[1] Université Paris-Saclay, CNRS, INRAE, Université Evry, Institute of Plant Sciences Paris-Saclay (IPS2), 91190, Gif sur Yvette, France
[2] Université Paris Cité, Institute of Plant Sciences Paris-Saclay (IPS2), 91190, Gif sur Yvette, France
[3] Université Paris-Saclay, AgroParisTech, INRAE, UMR MIA Paris-Saclay, 91120, Palaiseau, France
[4] Université Paris-Saclay, INRAE, CNRS, AgroParisTech, GQE-Le Moulon, 91190, Gif-Sur-Yvette,

The evolution of maize yields under water deficit conditions is of particular concern in the context of climate change and human population growth. However, the multiplicity and versatility of drought-response mechanisms make the design of new varieties a complex task that would greatly benefit from a better understanding of the genotype-phenotype relationship. The omnigenic model assumes that the entire genome contributes to complex traits, encouraging the consideration of small-effect SNPs that may explain missing heritability. Under such a hypothesis, we implemented here an innovative systems biology approach integrating the genetic determinism of molecular entities through the combination of genome-wide association studies and network inference. At each step of the integration process, a multi-environment mixed model was used to estimate the part of the genotype x water deficit interaction (GxW) variance captured by the genomic regions identified by our method. We applied our approach on a multi-omic dataset, including phenotypic, proteomic, and genomic data acquired from 254 maize hybrids grown under well-watered and water deficit conditions. Our results show that (i) QTLs underlying variations in protein abundance capture a part of the missing heritability of maize response to water stress response; (ii) there exists a synergy between the loci found in the two watering conditions and the loci associated with plasticity indices calculated from the two conditions; (iii) taking this synergy into account in our approach further increases the part of the GxW variance captured.
We found about 400 new loci capturing 89.4%, 66.5%, and 77.5% of the GxW variance of biomass, water use efficiency, and stomatal conductance, respectively, which brings a gain up to 20 points in captured GxW variances. Hence our results show that multi-omics data integration can be an efficient way to capture missing heritability for complex phenotypic traits and identify new candidate genes related to drought response.

Funding acknowledgement: LabEx Saclay Plant Sciences-SPS (ANR-17-EUR-0007, EUR SPS-GSR). The two French State grants from INRAE and C-Land (ANR-16-CONV-0003)

**T11**

# Transcriptional regulation of stress adaptation in maize: Identification and functional annotation

(submitted by Maggie Woodhouse <margaret.woodhouse@usda.gov>)
Full Author List: Hayford, Rita K.[1]; Haley, Olivia C[1]; Tibbs-Cortes, Laura[1]; Cannon, Ethalinda K[1]; Portwood II, John L[1]; Gardiner, Jack M[2]; Andorf, Carson M[1 3]; Woodhouse, Margaret R[1]
[1] USDA-ARS, Corn Insects and Crop Genetics Research Unit, Ames, IA 50011, USA
[2] Division of Animal Sciences, University of Missouri, Columbia, MO 65211, USA
[3] Department of Computer Science, Iowa State University, Ames, IA 50011, USA

Maize (Zea mays ssp. mays) is an essential grain crop cultivated for food, animal feed, fiber, and biofuel; therefore, maize yield improvements are necessary for future food security. Despite the importance of maize, various biotic and abiotic stresses negatively impact maize growth and development. Plants possess complex gene regulatory mechanisms influencing their responses to different stress factors. Chromatin Immunoprecipitation and Sequencing (ChIP-Seq) is a powerful method to understand transcription factor binding sites that regulate stress-responsive genes and map histone modifications controlling gene expression during stress. However, ChIP-Seq studies in maize during stress are under-explored. Although combining RNA-Seq and ChIP-Seq would provide a deeper understanding of gene regulation, such integrated study in maize is limited. We previously mapped high-quality RNA-Seq reads from publicly available datasets on B73 experiments. In this work, we aimed to map ChIP-Seq stress data from experiments from B73 imposed with stress to understand better how stress-response genes are regulated at the chromatin level. By integrating differentially expressed genes with chromatin regulatory mechanisms, we identified potential target genes in maize that provide valuable insights into stress responses. Comparing differentially marked peaks from ChIP-Seq data with RNA-Seq results allowed us to pinpoint stress-responsive genes and transcription factors. Our study also highlights the need for high-quality ChIP-Seq data in maize under stress conditions. Additionally, it demonstrates the importance of combining ChIP-Seq and RNA-Seq approaches to uncover transcriptional regulatory mechanisms governing stress responses in maize and other plant species.

Funding acknowledgement: United States Department of Agriculture (USDA)

**T12**

## A Rootless1 knockdown allele affects maize nodal root development, increasing rooting depth, nitrogen uptake efficiency, and grain production in the field

(submitted by Alexander Liu <aliu@danforthcenter.org>)
Full Author List: Liu, Alexander E[1 2]; Thiruppathi, Dhineshkumar[2]; Bray, Adam L[2]; Bagnall, George[2]; Morales, Elisa[2]; Lebow, Clara[2]; Topp, Christopher N[2]
[1] Washington University in Saint Louis, Saint Louis, MO, USA 63141
[2] Donald Danforth Plant Science Center, Saint Louis, MO, USA 63132

Nodal roots dominate the maize root system and are critical for nutrient acquisition. Changing nodal rooting patterns could help improve root system function; for example, the “Steep, Cheap, and Deep” paradigm posits that high nodal root production just above the soil line, but no higher, could increase nitrogen capture. Rootless1, a classic maize mutant, produces very few nodal roots aboveground. We previously identified a large indel in the promoter of *ZmRt1* that reduces expression which we hypothesize is responsible for the original Rootless1 phenotype. However, an Ac/Ds insertion allele of *ZmRt1*, named *rt1-2*, was observed to induce supernumerary nodal roots near the soil line before a precipitous decline at higher nodes in several maize backgrounds. We present a multi-year and multi-location analysis of changes to root system architecture due to the *rt1-2* nodal rooting phenotype and functional effects on plant growth and nitrogen status. Nitrogen contrast field experiments were performed in 2022, 2023, and 2024 in Missouri and Colorado comparing the *rt1-2* allele to the *ZmRt1* wild type allele in conventional and nitrogen limited conditions. Root system architecture was analyzed from excavated root crowns, 1m soil cores, and minirhizotrons. X-ray tomography analysis of excavated root crowns showed significant differences in root crown development. Deep soil cores revealed that plants with the rt1-2 allele have increased root length deeper in the soil column while minirhizotron data collected over the growing season showed differences in root system growth over time. To measure the functional impact of the observed changes in root system architecture, aboveground measures including shoot biomass, shoot nitrogen, and grain production were collected. Plants with the *rt1-2* allele had higher concentrations of shoot nitrogen, potentially leading to the observed increase in grain in both conventional and low nitrogen conditions when compared to the *ZmRt1* wild type allele, suggesting a positive influence on root resource capture efficiency.

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

**T13**

## Overexpression of *PSTOL1-like* genes increases maize root surface area and biomass under low and high phosphorus conditions

(submitted by Sylvia Morais de Sousa Tinoco <sylvia.sousa@embrapa.br>)
Full Author List: Negri, Barbara F.[1]; Maciel, Laiane S.[2]; Coelho, Antonio M.[3]; Barros, Beatriz A.[3]; Guimarães, Claudia T.[1 2 3]; Magalhães, Jurandir V.[1 2 3]; Tinôco, Sylvia M. S.[1 3]
[1] Federal University of São João del-Rei (UFSJ), São João del-Rei, MG, Brazil, 36301-158
[2] Federal University of Minas Gerais (UFMG), Belo Horizonte, MG, Brazil, 31270-901
[3] Embrapa Maize and Sorghum, Sete Lagoas, MG, Brazil, 35701-970

Low phosphorus (P) availability in soil is a significant limitation for crop production in tropical regions. The PHOSPHORUS-STARVATION TOLERANCE1 (OsPSTOL1) protein kinase enhances root surface area, P acquisition, and grain yield in rice under P-deficient conditions. Homologs of OsPSTOL1 in sorghum were identified through association mapping in two sorghum panels phenotyped for P uptake, root system morphology, and architecture in hydroponic systems, as well as grain yield and biomass accumulation under low-P conditions in Brazil and Mali. In maize and sorghum, candidate genes were co-localized with quantitative trait loci (QTL) associated with root morphology, dry weight, and grain yield under low P. To validate the function of these genes, the rice *OsPSTOL*1 (as a positive control) and its maize (*ZmPSTOL*3.06, *ZmPSTOL*8.02, and *ZmPSTOL8.05*_1) and sorghum (*Sb07g002840*, *Sb03g031690*, and *Sb03g006765*) homologs were cloned downstream of an ubiquitin promoter in the pMCG1005 vector, with the *Bar* gene serving as a selective marker. Genetic transformation of maize B104 embryos was performed using *Agrobacterium tumefaciens*. Homozygous transgenic events with a single copy of the transgene were selected, and those showing transgene overexpression were evaluated under low and high-P conditions. In a growth chamber, the events *Sb07g002840*, *Sb03g006765*, and *ZmPSTOL3.06* showed greater root length and total fine root surface area compared to the negative control (B104 transformed with an empty vector), under both low and high P-conditions. The Sb03g006765 event exhibited higher root and shoot dry weight under both low and high P. *ZmPSTOL8.02* presented a higher dry weight than the negative control only under low P, while *Sb07g002840* had a higher shoot dry weight under high P. In the greenhouse, the *Sb03g006765*, *ZmPSTOL8.02*, and *ZmPSTOL8.05_1* events showed increased shoot dry weight under low P, while *OsPSTOL1*, *Sb07g002840*, and *Sb03g006765* exhibited higher shoot dry weight under high P. For root dry weight under low P, *Sb03g006765*, *ZmPSTOL8.02*, and *ZmPSTOL8.05_1* were superior to the negative control. Under high P, all events except *ZmPSTOL8.02* outperformed the negative control. Overexpression of the *PSTOL*1 homologs significantly improved vegetative growth and root surface area, demonstrating that these genes function similarly to *OsPSTOL*1 in rice.

Funding acknowledgement: Embrapa, GCP, Capes, CNPq

**T14** @StephEMartinez

## Delayed divisions and cell elongation defects influence plant growth in katanin mutants

(submitted by Stephanie Martinez <smart046@ucr.edu>)
Full Author List: Martinez, Stephanie E[1]; Allsman, Lindy A[1]; Ceballos, Ian[1]; Irahola, Cassandra[1]; Lau, Kin H[2]; Wright, Amanda J[3]; Weil, Clifford[4 5]; Rasmussen, Carolyn G[1]
[1] Department of Botany and Plant Sciences, Center for Plant Cell Biology, Institute for Integrative Genome Biology, University of California Riverside, Riverside, CA 92521, USA
[2] Bioinformatics and Biostatistics Core, Van Andel Institute, Grand Rapids, MI 49503, USA
[3] Unaffiliated, Lewisville, TX, USA
[4] Purdue University, West Lafayette, IN 47907, USA
[5] Division of Molecular and Cellular Biosciences, National Science Foundation, Alexandria, Virginia 22314, USA

Microtubule dynamics and organization influence cell shape and cell division plane orientation. One protein complex involved in this process is KATANIN, a microtubule severing AAA ATPase hexameric complex composed of catalytic p60 and regulatory WD-40-containing p80 subunits. In Zea mays, two genes encode KATANIN (p60), and mutants have been identified called discordia3a (dcd3a) and dcd3b. Live cell imaging showed reduced microtubule severing frequency in dcd3a-2 dcd3b double mutants compared to wild-type siblings. Similar to katanin mutants in other organisms, dcd3a-2 dcd3b mutants have decreased plant height. To determine if defects in cell elongation cause the reductions in leaf area, cell dimensions were measured. When compared to wild-type siblings, dcd3a-2 dcd3b mutants have smaller epidermal cells, as seen by significant reduction in cell area and length to width ratios, but these small cells are not the primary cause of reduced leaf area. Instead, the dcd3a dcd3b mutants have approximately four times fewer cells. To determine if cell division delays cause fewer cells, we measured cell division progression in dcd3a-2 dcd3b and wild-type siblings. There was no significant delay from metaphase until the completion of cytokinesis. However, a significant decrease in the proportion of cells in late G2 and prophase in dcd3a-2 dcd3b double mutant suggests delays in mitotic entry. Therefore, a combination of cell elongation defects and delayed mitotic entry generates small mutant plants.

Gene / Gene Models described: *ktn2, clt1*; Zm00001eb156560, Zm00001eb360490
Funding acknowledgement: National Science Foundation (NSF)

**T15**

## The EPF-ERECTA ligand-receptor pairs regulate maize shoot and inflorescence architecture in coordination with CLAVATA pathway in maize

(submitted by Fang Xu <fxu@sdu.edu.cn>)
Full Author List: Liu, Xiao[1]; Jackson, David[2]; Xu, Fang[1]
[1] Shandong University, Qingdao, Shandong, China, 266237
[2] Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, USA,11724

In maize, several key yield-related traits are associated with meristem activity, regulated by CLE peptide signals recognized by receptor-like kinases or proteins in the CLAVATA-WUSCHEL pathway. In this study, we identified three additional receptor-like kinases, ZmER1 (ZmERECTA), ZmER2 and ZmERL, and their cognate ligands, ZmEPFs (EPIDERMAL PATTERNING FACTOR), which play critical roles in regulating meristem activity and are essential for plant architecture and ear development. Utilizing CRISPR/Cas9 gene-editing technology, we generated ZmERs knockout mutants. The *Zmer1* mutant displayed a compact plant architecture, an enlarged inflorescence meristem (IM), and increased kernel row number, while *Zmer2* and *Zmerl* mutants showed no obvious defects. Double mutants of *Zmer1; Zmer2* and *Zmer1; Zmerl* and triple mutants showed more severe plant architecture defects and larger IM meristem size compared to *Zmer1* single mutant, suggesting redundant roles of *ZmERs* genes, with *ZmER1* playing a dominant role. Microscale thermophoresis experiments showed that ZmER1 binds specifically to several ZmEPF peptides but not the CLE peptides. CRISPR knock-outs of five ZmEPF genes revealed that single mutant of ZmEPF showed no obvious phenotypes, but higher-order mutants exhibited significantly enlarged IM, suggesting functional redundancy among the ZmEPFs. Notably, unlike the *Zmers* mutant with both plant architecture and ear phenotype, quintyple *Zmepfs* displayed no plant architecture defect, implying a specific role for ZmEPFs in inflorescence meristem regulation and a function uncoupling between the receptor and its ligand. Interestingly, ZmER1 directly interacts with ZmCRN, a key signaling component of the CLV pathway, and *Zmwus1* mutant suppressed the enlarged inflorescence meristem caused by *Zmer1* mutation. These findings suggest that ZmERs regulate meristem development by integrating with the CLV-WUS pathway. This study provides new insights into ZmER-mediated regulation of plant and ear architecture and identifies candidate genetic targets for breeding high-yielding maize varieties through optimizing the plant and ear architecture.

Funding acknowledgement: National Science Foundation of China

**T16**

## Catalytic and non-catalytic TREHALOSE-6-PHOSPHATE SYNTHASES (TPSs) interact with RAMOSA3 to control maize development

(submitted by Thu Tran <tran@cshl.edu>)
Full Author List: Tran, Thu[1]; Claeys, Hannes[2]; Michalski, Kevin[1]; Boumpas, Panagiotis[3]; Williams, Z'Dhanne[4]; Sheppard, Samatha[1]; Vi, Son[5]; Chou, Tsung Han[1]; Furukawa, Hiro[1]; Jackson, David[1]
[1] Cold Spring Harbor Laboratory, 1 Bungtown Road, Cold Spring Harbor, NY11724, USA
[2] Inari, Industriepark 7A, 9052 Zwijnaarde, Belgium
[3] Heidelberg University, Centre for Organismal Studies (COS) Heidelberg, Department of Developmental Biology, Heidelberg, Germany
[4] Salivary Disorders Unit, National Institute of Dental and Craniofacial Research, National Institute of Health, Bethesda, MD, USA
[5] Faculty of Biotechnology, Chemistry and Environmental Engineering, PHENIKAA University, Yen Nghia, Ha Dong, Hanoi 12116, Vietnam

Trehalose-6-phosphate (T6P) is a key regulator of plant signaling networks, and coordinates growth by influencing carbon allocation, stress responses, architecture, and developmental transitions. T6P is the intermediate of trehalose biosynthesis mediated by T6P-synthases (TPSs) and T6P-phosphatases (TPPs). Plants harbor small families of *TPS* and *TPP* genes; while all TPPs are catalytic active, most plant TPSs are non-catalytic, suggesting they have regulatory functions. Here, we show that non-catalytic TPSs form a tripartite complex with catalytic TPSs and TPPs to control enzymatic activity and development.Maize mutant *ramosa3* (*ra3*) increases inflorescence branching and RA3 encodes a catalytic TPP. To investigate RA3 molecular mechanism, we screened for interactors, and found that it interacts with two non-catalytic TPS1, TPS12. *tps1* and *tps12* mutants enhance *ra3* phenotypes, suggesting their interaction is biologically significant. Interestingly, we found that TPS1 also interacts with the two maize catalytic TPS11 and TPS14. We knocked out these genes using CRISPR-Cas9, and the double mutants are embryo lethal. However, reducing active TPS expression in the *ra3; tps1; tps12* triple mutant background modifies its phenotype, supporting the idea interaction between three classes of proteins.To ask if TPS-TPP interactions affect enzyme activity, we performed a coupled enzyme assay, and found that the non-catalytic TPS1 stimulated the activity of RA3 and TPS14. This result suggests that RA3, TPS1, and TPS14 form a complex, and we confirmed after purifying the three proteins from insect cells. We used AlphaFold to predict that the TPS domains of TPS1 and TPS14 initiate this complex. To confirm this prediction, we co-expressed TPS1 and TPS14 and visualized heterotetramer complex formation by cryo-electron microscopy. From these results, we propose that the TPS1 TPS14 heterotetramer is important for both enzymatic activity and complex formation. These results provide insights into the structural basis and stoichiometry of TPS-TPP interactions, which have not been studied in any organism.In summary, we show a maize TPP (RA3) functions in a complex with both non-catalytic and catalytic active TPSs, and the non-catalytic TPS stimulates the activity of the active enzymes. Our research provides insights for the first time into the combined activity of the two major trehalose gene classes in plant development.

Funding acknowledgement: National Science Foundation (NSF)

**T17**

## Exploring the effects of ethylene-related transcription factors during maize shoot development

(submitted by Alejandro Aragon Raygoza <jaaragon@iastate.edu>)
Full Author List: Aragon-Raygoza, Alejandro[1 2]; Galli, Mary[3]; Best, Norman[4]; Unger-Wallace, Erica[2]; Satterlee, James[5]; Beeler, Charlie[2]; Yen, Jeffrey[5]; Rasey, Oliver[1]; Darst, Faith[1]; Vollbrecht, Erik[2]; Gallavotti, Andrea[3]; Strable, Josh[1 2]
[1] Department of Molecular and Structural Biochemistry, North Carolina State University
[2] Department of Genetics, Development and Cell Biology, Iowa State University
[3] The Waksman Institute of Microbiology, Department of Plant Biology, Rutgers University
[4] Plant Genetics Research Unit, USDA-ARS
[5] Plant Biology Section, Cornell University

The plant hormone ethylene is a key developmental regulator that acts largely to restrict growth. Ethylene signaling and response are well-studied in eudicots; in contrast, our understanding of ethylene function in cereal crops is markedly limited. Here, we characterized mutations in maize *ETHYLENE INSENSITIVE3*(*EIN3*)-*LIKE1* (*ZmEIL1*) and *ZmEIL9* genes that encode co-orthologs of the Arabidopsis *EIN3/EIL1* hub transcription factors. By stacking independently derived mutations in *ZmEIL1* and *ZmEIL9*, we uncovered vegetative shoot phenotypes associated with mis-regulated growth that are distinct from Arabidopsis *ein3;eil1* mutants. We show that *Zmeil1;Zmeil9* double mutants are insensitive to the ethylene precursor ACC in separate germination and growth assays. By utilizing single-cell transcriptomics, we identified tissue- and cell-specific genetic signatures in *Zmeil1;Zmeil9* and normal shoots with or without exogenous ACC. We found that differentially expressed genes in hormone pathways are highly correlated with altered accumulation of hormone pathway intermediates in *Zmeil1;Zmeil9* shoot apices. Gene ontology analysis revealed enrichment of abiotic stress response categories in *Zmeil1;Zmeil9* upregulated genes, whereas genes encoding translational machinery were downregulated in mutant shoot apices. Translation was enriched in genes upregulated by response to ACC, while developmental process and response to stimulus were significant categories in ACC-downregulated genes. We leveraged DAP-seq to identify genome-wide binding positions of ZmEILs. Approximately 85% of ZmEIL1 and ZmEIL9 bound filtered-genic regions overlap, suggesting co-regulation of a large portion of putative target genes, including a proportion of genes that were identified as differentially expressed in our single-cell data. Genes directly bound and modulated by ZmEILs include *ZAR* and *RTL* family members that are negative regulators of ethylene signaling, and that also have important roles in organ growth. Our study shows that *ZmEIL* genes are central regulators of shoot growth and, by resolving cellular and tissue heterogeneity of ethylene response, significantly advances our understanding of ethylene signaling in plants.

Funding acknowledgement: National Science Foundation (NSF), Startup funds

**SESSION 8 – REPRODUCTION / KEYNOTE**
Chair: Cinta Romay Saturday, March 8. 7:00 PM – 8:20 PM

**T18**

## Understanding the molecular mechanism of parthenogenesis in cereals

(submitted by Xixi Zheng <Xixi.zheng@ur.de>)
Full Author List: Zheng, Xixi[1]; Tornero, Maria Flores[1]; Schwartz, Uwe[2]; Dresselhaus, Thomas[1]
[1] Cell Biology and Plant Biochemistry, University of Regensburg, 93053 Regensburg, Germany
[2] Computational Core Unit, University of Regensburg, 93053 Regensburg, Germany

Parthenogenesis, describes spontaneous embryogenesis from an unfertilized egg cell and thereby generates offspring genetically identical to the mother plant, and is a key component of apomixis (asexual reproduction through seeds). Investigating parthenogenesis in crop plants not only has high potentials to immediately fix desired traits including heterosis and thus would create great economic values, but would also help to understand how egg cell fate is determined for embryogenesis initiation. The underlying mechanisms of parthenogenesis remain poorly understood. Here, we use the apomictic grass *Tripsacum dactyloides* to address these questions. As the closest wild relative of maize, Tripsacum is sexually reproducing as a diploid, but all polyploids display apomixis via parthenogenesis. Wecollected egg cells from diploid and tetraploid Tripsacum lines to compare their gene expression. We observed that parthenogenetic eggs possess relatively specific cell cycle gene expression pattern that confers division potentials. Transcriptional reprogramming considerably contributes via both ON/OFF and differential regulation modes primarily involved in cell differentiation and auxin signaling. Parthenogenesis and zygotic genome activation share similar gene expression alterations associated with RNA metabolisms at both transcriptional (e.g. via ZmBBM1) and post-transcriptional (e.g. via RNase exonuclease) levels. These genes are highly expressed in parthenogenetic eggs but completely silenced in sexual eggs. We are currently characterizing the funtion(s) of candidate genes via creation ofectopic sexual egg cell-expression lines and knock-outs in maize. We found both ectopic expression of *ZmBBM1* or knock out can induced twin embryos. ZmBBM1-mEGFP fluorescence are abundantly detected in embryonic pro-vascular and root systems. The ultimate goal of this research is to gain a mechanistic understanding of parthenogenesis and embryogenesis initiation in cereals and to utilize the knowledge generated to contribute and improve the production of haploid maize lines and/or clonal seeds.

Funding acknowledgement: Alexander von Humboldt Foundation

**T19**

## Heat treatment and UBA2 fusions enhance LbCas12a genome editing activity during haploid induction

(submitted by Rachel Egger <rachel.egger@syngenta.com>)
Full Author List: Liang, Dawei[1]; Zhu, Fugui[1]; Wei, Juan[1]; Guo, Huan[1]; Zhang, Yuguo[1]; Green, Julie[2]; Ji, Yun[1]; Jin, Huaibing[1]; Zhang, Xiujuan[1]; Liu, Yubo[1]; Zhang, Yu[1]; Wang, Han[1]; Chen, Xi[1]; Kelliher, Tim[2]; Egger, Rachel[2]
[1] State Key Laboratory of Crop Germplasm Innovation and Molecular Breeding, Syngenta Biotechnology (China) Co., Ltd, Beijing, China
[2] Syngenta Seeds Research, Research Triangle Park, NC, USA

Haploid induction (HI) coupled with genome editing, known as HI-Edit, has emerged as a powerful tool for direct genomic modification of commercial crop varieties including maize, eliminating the need for direct variety transformation of CRISPR machinery. However, the efficiency of this method has been limited by relatively low haploid editing rates (HER). In this study, we report significant improvements to HI-Edit efficiency through the application of post-pollination heat treatment, LbCas12a expression with a male-gamete-specific promoter, and the use of a LbCas12a-UBA2 fusion protein to promote protein stability during HI. Our results demonstrate that heat treatment increased haploid editing rates by 10-fold, providing a simple yet effective means to enhance the yield of edited haploid plants. Furthermore, we found that UBA2 fusion can further increase haploid editing rate by 4-fold, offering an additional strategy to boost editing efficiency. The combination of heat treatment and UBA2 fusion resulted in a cumulative 16-fold increase in haploid editing rates, significantly outperforming traditional HI-Edit protocols. In this experiment, an average of 25% of all haploids were successfully edited across two testers at a single gRNA target site. Our findings provide a robust framework for expanding the scalability of this technology in maize and potentially across diverse plant species, enhancing the efficiency of HI-Edit and accelerating crop improvement.

Funding acknowledgement: Syngenta Seeds

**T20**

## Molecular evolution of the Ga reproductive barriers in maize and related species

(submitted by Elli Cryan <epcryan@ucdavis.edu>)
Full Author List: Cryan, Elli[1 2 3]; Phinney, Garnet[2]; Seetharam, Arun S.[4 5]; Evans, Matthew M.S.[6]; Kellogg, Elizabeth A.[7 8]; Zhan, Junpeng[7]; Meyers, Blake C.[1 7 9 10]; Kliebenstein, Daniel J.[1 3 10]; Ross-Ibarra, Jeffrey[2 3 10]

[1] Plant Sciences, UC Davis; Davis, CA
[2] Evolution and Ecology, UC Davis; Davis, CA
[3] Center for Population Biology, UC Davis; Davis, CA
[4] Ecology, Evolution and Organismal Biology, Iowa State University; Ames, IA
[5] Genetics, Development and Cell Biology, Iowa State University; Ames, IA
[6] Plant Biology, Carnegie Institution for Science; Stanford, CA
[7] Donald Danforth Plant Science Center; St. Louis, MO
[8] Arnold Arboretum of Harvard University; Boston, MA
[9] Plant Science & Technology, University of Missouri - Columbia; Columbia, MO
[10] Genome Center, UC Davis; Davis, CA

Three cross-incompatibility loci, Teosinte crossing barrier1 (Tcb1), Gametophytic factor1 (Ga1), and Ga2, each play a key role in preventing hybridization between incompatible populations and are proposed to maintain the barrier between domesticated maize and wild teosinte subspecies. Each locus encodes both a silk-active and a matching pollen-active pectin methylesterase (PME). To investigate the diversity and molecular evolution of these gametophytic factor loci, we identified existing and improved models of the responsible genes in a new genome assembly of maize line P8860 that contains active versions of all three loci. We then examined fifty-two assembled genomes from seventeen species to classify haplotype diversity and identify sites under diversifying selection during the evolution of these genes. We show that Ga2, the oldest of these three loci, was duplicated to form Ga1 at least 12 million years ago. Tcb1, the youngest locus, arose as a duplicate of Ga1 before or around the time of diversification of the Zea genus. We find evidence of positive selection during evolution of the functional genes at an active site in the pollen-expressed PME and predicted surface sites in both the silk- and pollen-expressed PMEs. The most common allele at the Ga1 locus is a conserved ga1 allele (ga1-Off), which is specific haplotype containing three full-length PME gene copies, all of which are non-coding due to conserved stop codons and are between 610 thousand and 1.5 million years old. We show that the ga1-Off allele likely generates 24-nt siRNAs in developing pollen-producing tissue, and these siRNAs map to functional Ga1 alleles. In crossing experiments, the ga1-Off allele was associated with reduced strength of the typically dominant functional alleles for the Ga1 and Tcb1 barriers. Taken together, this seems to be an example of paramutation functioning at a locus controlling a reproductive barrier.

Gene / Gene Models described: *ga1*, *ga2*, *tcb1*, *ZmPME3*; Zm00001eb167600, Zm00001eb239230
Funding acknowledgement: National Science Foundation (NSF), United States Department of Agriculture (USDA), UC Davis Plant Sciences, UC Davis Plant Biology

**T21**

## Long-distance retrotransposons direct variable gene imprinting in maize

(submitted by Qi Li <qi.li@zmbp.uni-tuebingen.de>)
Full Author List: Li, Qi[1 2]; Zheng, Xixi[1 3]; Li, Changsheng[1 4]; Wu, Yongrui[1]
[1] National Key Laboratory of Plant Molecular Genetics, CAS Center for Excellence in Molecular Plant Sciences, Shanghai Institute of Plant Physiology & Ecology, Chinese Academy of Sciences, Shanghai 200032, China
[2] Center for Plant Molecular Biology, University of Tübingen, Tübingen 72076, Germany
[3] Cell Biology and Plant Biochemistry, Institute of Plant Sciences, University of Regensburg, Regensburg 93053, Germany
[4] The National Engineering Laboratory of Crop Stress Resistance Breeding, Anhui Agricultural University, Hefei 230036, China.

Genomic imprinting dictates the preferential expression of parental alleles based on their origin, rather than dosage. Despite decades of research, understanding how imprint control arises or is removed at specific loci through regulatory regions, and how to shape the widespread intra-species imprint variability, remains limited. Here, we developed a genetic screening system utilizing a classic maize endosperm mutant that exhibits maternally inherited dominant-negative effects due to conserved paternal imprints on the causal locus. By screening an inbred line panel and sequencing the genome of a specific inbred, we identified rare haplotypes that bypass imprinting. We discovered that the conserved locus-specific imprinting is maintained by a *Flip* retrotransposon located hundred kilobases upstream, which has persisted throughout maize domestication. The *Xilon-Diguus* and *Cinful-Zeon* impede imprinting at the locus by inserting into the *Flip*, forming nested retrotransposon structures. Our findings shed light on how single, distant retrotransposons can both preserve and perturb gene imprinting through proximal-distal interactions. Our research highlights the pivotal role of hidden, often overlooked distal sequences—particularly transposable element nesting—in driving fluctuations in gene expression within natural populations.

Gene / Gene Models described: *fl3*; GRMZM2G006585

**T22**

## Deciphering epigenetic and genetic alterations in a DNA methylation mutant through successive generations of self-fertilization in maize

(submitted by Xi Cheng <xicheng@ufl.edu>)
Full Author List: Cheng, Xi[1 2]; Zhao, Meixia[1 2]; Lee, Gwonjin[3]
[1] Plant Molecular and Cellular Biology Program, University of Florida, Gainesville, FL 32610, USA
[2] Department of Microbiology and Cell Science, University of Florida, Gainesville, FL 32611, USA
[3] Department of Biology, West Virginia State University, Institute, WV 25112, USA

Enhancing crop productivity in the face of global challenges relies on a deeper understanding of the genetic and epigenetic mechanisms governing plant growth and development. In particular, how plants maintain genome stability and regulate gene expression under epigenetic changes is becoming increasingly critical. Our research focuses on maize plants mutant for a small RNA biosynthesis pathway gene, named *Mop1* (*Mediator of paramutation1*). These *mop1* mutants exhibit increasingly severe phenotypes related to growth and development after several generations of self-fertilization in inbred plants, a phenomenon not observed in wild-type plants. Our DNA methylation analysis of *mop1* mutants homozygous for three generations revealed that while genome-wide CG and CHG (where H = A, T, or C) methylation levels remained relatively stable, CHH methylation was rapidly removed in the first-generation mutants, with no further reductions in subsequent generations. Interestingly, despite the global stability of CG and CHG methylation, the numbers of both hyper- and hypomethylated CG and CHG differentially methylated regions (DMRs) increased dramatically over successive generations, indicating substantial local changes. While hypermethylated DMRs accounted for only 10% of the total, a significant proportion of these, particularly CHG hyper-DMRs, were enriched within introns of genes overlapping long interspersed nuclear elements (LINEs). To validate our findings and test hypotheses concerning the role of polymorphic transposable elements (TEs) in these epigenetic changes, we analyzed mop1 mutants homozygous for multiple generations in the Mo17 background. The results in Mo17 mirrored those in B73, with increasing DMRs in non-CHH contexts and similar epigenetic changes, indicating that these patterns are not specific to a particular maize genotype. Our next steps involve exploring gene expression, chromatin accessibility, and histone modifications in these plants, focusing on genes or regions at or near those DMRs. By uncovering the interplay between DNA methylation, differential expression of genes or TEs, and chromatin structures, we aim to elucidate the genetic and epigenetic mechanisms driving maize development. These insights hold practical significance, enabling innovative epigenetic engineering approaches to enhance maize productivity and performance while advancing the understanding of plant epigenetic regulation.

Gene / Gene Models described: *mop1*; Zm00001eb080370
Funding acknowledgement: National Science Foundation (NSF), University of Florida, Startup Fund to Dr. Meixia Zhao

**T23**

## Replication timing uncovers a novel two-compartment arrangement of maize interphase euchromatin

(submitted by Hafiza Sara Akram <ha20be@fsu.edu>)
Full Author List: Akram, Hafiza Sara[1]; Wear, Emily E.[2]; Mickelson-Young, Leigh[2]; Turpin, Zach M.[1]; Thompson, William F.[2]; Hanley-Bowdoin, Linda K.[2]; Concia, Lorenzo[3]; Bass, Hank W.[1]
[1] Department of Biological Science, Florida State University, Tallahassee, FL, USA
[2] Plant and Microbial Biology, North Carolina State University, Raleigh, NC, USA
[3] Texas Advanced Computing Center, University of Texas, Austin, TX, USA

The time within S phase when a given genomic region replicates is measurable and referred to as Replication Timing (RT). We previously proposed a "mini-domain" chromatin fiber RT model for maize euchromatin's spatial architecture. 3D quantitative cytology showed that euchromatin is subdivided into two compartments during S-phase, distinguished by chromatin condensation and replication timing: Early-S and Middle-S. A key gap remains in understanding whether this compartmentalization is a general feature throughout the cell cycle, which could greatly impact our knowledge of genome architecture and gene regulation. To investigate this, we conducted two orthogonal assays, Hi-C for genome-wide interaction data and 3D FISH for direct visualization of chromatin organization. The Hi-C-derived eigenvalues and insulation scores significantly concord with Early-S and Middle-S regions. Early-S regions showed negative insulation scores with more long-range contacts, whereas Middle-S regions displayed the opposite, positive insulation scores with fewer long-range contacts. Compared to Middle-S regions, the Early-S regions showed much stronger correlations with epigenomic signatures of open and transcriptionally active chromatin. Oligo FISH painting with RT-specific probes demonstrated that Early-S and Middle-S regions occupied adjacent but largely non-overlapping nucleoplasmic regions throughout all stages of interphase, including G1. These findings validate our model while establishing that the global "A" compartment of euchromatin actually consists of two spatially and epigenetically distinct substructures – now referred to as Early-S and Middle-S compartments. Given the strong correlation between replication timing and Hi-C data from root tips, we examined the conservation of Hi-C architecture in both root tip and earshoot tissues and found them to be remarkably similar. Overall, our findings highlight the importance of replication timing as a conserved feature of chromatin architecture, reflecting broader principles of genome organization.

Funding acknowledgement: National Science Foundation (NSF)

**T24**

## Determination of genetic and epigenetic regulations of meiotic recombination during domestication in maize

(submitted by Akwasi Yeboah <akwasiyeboah@ufl.edu>)
Full Author List: Yeboah, Akwasi[1]; Lee, Gwonjin[2]; Flint-Garcia, Sherry A.[3]; Zhao, Meixia[1]
[1] University of Florida, Gainesville, FL 32603
[2] West Virginia State University, Institute, WV 25112
[3] University of Missouri, Columbia, MO 65211

Meiotic recombination involves the exchange of genetic material between homologous chromosomes, playing a key role in evolution and genetic diversity. Meiotic crossovers (COs) are not evenly distributed on chromosomes; instead, they are enriched in hotspots controlled by genetic and epigenetic factors. In maize, meiotic recombination has significantly contributed to maize domestication. However, the specific mechanisms underlying this process remain largely unexplored. This study aims to elucidate the genetic mechanisms driving meiotic recombination during maize domestication and to compare how meiotic recombination differs between sexes. Our investigation encompasses a diverse set of maize lines, including 5 teosintes, 7 landraces, and 15 cultivars. These lines were selected because they maximize genetic diversity and thus are most likely to show variation in recombination. Our results reveal that the teosinte line BK4 and the landrace MR19 exhibited longer genetic distances and higher crossover numbers, whereas BK1 and MR11 showed the lowest, compared to the other teosinte and landrace lines. Of the 27 total lines including cultivars, BK4 and MR19 were still among the highest in crossover numbers, while BK1 and CML103 showed the lowest. CO hotspot analysis revealed that most hotspots were located at the distal ends of each chromosome across all lines. Additionally, our results reveal significant differences in CO number and recombination rate between sexes, and these differences were dependent on different maize genetic backgrounds. Furthermore, analysis of distance CoC showed that teosinte lines exhibited higher, though not statistically significant, levels of CO interference compared to other maize lines. In our ongoing research, we are collecting immature anthers from maize plants exhibiting extreme CO numbers and recombination rates for transcriptome and DNA methylation analysis. This work aims to uncover the genetic and epigenetic factors underlying meiotic recombination in maize, thereby enhancing our understanding of this crucial biological process.

Funding acknowledgement: National Science Foundation (NSF)

**T25**

## Deciphering genetic architecture of stalk lodging resistance using high-density phenotype map in maize

(submitted by Bharath Kunduru <bkundur@clemson.edu>)
Full Author List: Kunduru, Bharath[1]; Bokros, Norbert[2 3]; Tabaracci, Kaitlin[4]; Kumar, Rohit[1 5]; Brar, Manwinder[1]; Machado e Silva, Caique[6 7]; Oduntan, Yusuf[4]; DeKold, Joseph[4]; Woomer, Joseph[3]; Bishop, Rebecca[1]; Verges, Virginia[3]; Stubbs, Christopher[4 8]; Morota, Gota[6]; McMahan, Christopher[9]; Robertson, Daniel[4]; DeBolt, Seth[3]; Sekhon, Rajandeep[1]
[1] Department of Genetics and Biochemistry, Clemson University, SC, USA
[2] Center for Quantitative Genetics and Genomics, Aarhus University, Aarhus, Denmark
[3] Department of Horticulture, University of Kentucky, KY, USA
[4] Department of Mechanical Engineering, University of Idaho, ID, USA
[5] Department of Plant and Environmental Sciences, Clemson University, SC, USA
[6] School of Animal Sciences, Virginia Polytechnic Institute and State University, VA, USA
[7] Department of Agronomy, Federal University of Viçosa, Minas Gerais, Brazil
[8] School of Computer Sciences and Engineering, Fairleigh Dickinson University, NJ, USA
[9] School of Mathematics and Statistical sciences, Clemson University, SC, USA

Stalk lodging undermines crop productivity and causes global annual losses of at least 6 billion USD in maize (Zea mays L.). Poor understanding of plant phenotypes associated with stalk lodging, referred to as intermediate phenotypes, and lack of standardized phenotyping protocols impeded the efforts to enhance genetic resolution of stalk lodging resistance. We generated a multi-environment high-density phenotype map of 15 morphological, geometric, structural, and biomechanical phenotypes on 31,200 stalks of a maize inbred panel, examined the impact of these phenotypes on stalk lodging resistance, and identified underlying genetic loci. Preserving field location of individual plants allowed us to account for spatial effects and refine the phenotype data for statistical analyses. Phenotype analyses revealed significant variation across environments indicating a substantial role of G×E on the phenotypic continuum of intermediate phenotypes. Predictive analytics with machine learning revealed major and minor diameters of internodes and plant height as the key predictors of stalk flexural stiffness. Intermediate phenotypes measured on the lower-most elongated internode showed stronger genetic correlation with stalk flexural stiffness as compared to those measured on the primary ear-bearing internode. Most intermediate phenotypes exhibited low to moderate heritability indicating low genetic tractability and complex genetic inheritance. Genome wide association analyses of the intermediate phenotypes showed candidate genes involved in cell division, plant and ear height, electron transport, membrane structure and transport, oxidoreductase activity, transcription factors, etc. Remarkably, a large proportion of the significant SNPs identified were nested in the non-coding regions of the genome and certain SNPs were shared between different phenotypes indicating pleiotropic regulation of stalk lodging resistance. We are currently evaluating the role of selected candidate genes for their role in stalk lodging resistance in maize.

Funding acknowledgement: National Science Foundation (NSF)

**T26**

## Plasticity and fitness trade-offs in switchgrass revealed by open science and citizen science data

(submitted by Laura Tibbs-Cortes <laura.tibbs-cortes@usda.gov>)
Full Author List: Tibbs-Cortes, Laura E[1 2]; Jewell, Jeremy[3]; Bartley, Laura[3]; Swaminathan, Kankshita[4]; Andorf, Carson[1 2]; Juenger, Thomas[5]; Yu, Jianming[2]; Li, Xianran[3 6]
[1] USDA-ARS Corn Insects and Crop Genetics Research Unit, Ames, IA, USA 50011
[2] Iowa State University, Ames, IA, USA 50011
[3] Washington State University, Pullman, WA, USA 99164
[4] HudsonAlpha Institute for Biotechnology, Huntsville, AL, USA 35806
[5] University of Texas at Austin, Austin, TX, USA 78712
[6] USDA-ARS Wheat Health, Genetics, and Quality Research Unit, Pullman, WA, USA 99164

Switchgrass (*Panicum virgatum*), a member of the Panicoideae subfamily which includes maize, is a perennial grass native to the North American tallgrass prairie that today is grown as a biomass crop for bioenergy. Switchgrass provides a unique opportunity to study adaptation and phenotypic plasticity across a large environmental gradient because it not only has a wealth of open science genomic and phenotypic data available from multi-environment trials (METs), but is also widely observed by citizen scientists across its extensive native range. Based on thousands of research-grade observations from the citizen science repository iNaturalist, we identified a conserved trend in flowering time across latitudes. We then applied the CERIS-JGRA algorithm and QTL mapping to open science MET data in order to identify specific genetic and environmental factors influencing flowering time as well as biomass and over-winter survival rates. We found that these traits were strongly influenced by temperature and identified three major candidate genes underlying this plastic response to the environment. Candidate genes are currently being validated by CRISPR. Intriguingly, by aligning the switchgrass proteome to the maize proteome, we found that two of these three candidate genes were also recently published as candidate genes underlying plasticity in maize. Alternative haplotypes at these loci differ substantially in their degree of phenotypic plasticity in response to identified environmental cues, resulting in fitness trade-offs across the native range of switchgrass. We combined our CERIS-JGRA models with past weather data and future climate projections to model shifts in the distribution of switchgrass populations over time. Finally, by uncovering and resolving apparently opposite trends in flowering time between the citizen science and MET data, we provide insights for interpretation of these data types while leveraging their complementary strengths.

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

**T27**

## Inactivation of a lysine-histidine transporter-1 gene confers southern leaf blight resistance in maize

(submitted by Qin Yang <qyang@nwafu.edu.cn>)
Full Author List: Wang, Yapeng[1]; Huang, Xiaojian[1]; Tian, Yuan[1]; Wang, Zhe[1]; Tai, Huanhuan[1]; Song, Weibin[2]; Balint-Kurti, Peter[3 4]; Yang, Qin[1]
[1] State Key Laboratory for Crop Stress Resistance and High-Efficiency Production, College of Agronomy, Northwest A&F University, Yangling 712100, P. R. China
[2] National Maize Improvement Center, Department of Plant Genetics and Breeding, China Agricultural University, Beijing 100193, P. R. China
[3] Department of Entomology and Plant Pathology, North Carolina State University, Raleigh, NC 27695, USA
[4] Plant Science Research Unit, USDA-ARS, Raleigh 27695, NC, USA

Southern leaf blight (SLB) is one of the most serious foliar diseases in maize worldwide. *qSLB*$_{6.01}$ is a major quantitative trait locus conferring recessive resistance to SLB. Through map-based cloning, ethyl methanesulfonate mutagenesis, and CRISPR-Cas9 editing, we demonstrate that a lysine-histidine transporter-1 (*ZmLHT1*) gene at *qSLB*$_{6.01}$ confers quantitative susceptibility to SLB. A 354 bp insertion in the *ZmLHT1* coding region in the resistant parental line NC292 creates a truncated protein, resulting in enhanced disease resistance to SLB. Targeted mutation of *ZmLHT1* leads to robust SLB resistance without affecting other important agronomic traits. *ZmLHT1* encodes a plasma membrane-localized broad-spectrum amino acid transporter. Transcriptome profiling reveals that genes involved in plant secondary cell wall biosynthesis were strongly induced in leaves of the *zmlht1* mutant after *Cochliobolus heterostrophus* infection, whilst cell redox homeostasis-related genes were highly expressed in wildtype plants. Moreover, we present evidence that ZmLHT1 reduces ROS deposition and inhibits secondary cell wall thickening during *C. heterostrophus* infection. Our findings may aid in disease resistance breeding through marker-assisted selection or genome editing while balancing growth-defense tradeoffs in maize.

Funding acknowledgement: National Natural Science Foundation of China (#32272089), the Key Research and Development Program of Shaanxi (#2024NC2-GJHX-33).

**T28**

## Regulation of heterosis-associated gene expression complementation in maize hybrids

(submitted by Marion Pitz <mpitz@uni-bonn.de>)
Full Author List: Pitz, Marion[1]; Baldauf, Jutta A.[1]; Piepho, Hans-Peter[2]; Yu, Peng[3]; Schoof, Heiko[4]; Mason, Annaliese S.[5]; Li, Guoliang[6]; Hochholdinger, Frank[1]
[1] Institute of Crop Science and Resource Conservation, Crop Functional Genomics, University of Bonn, 53113 Bonn, Germany
[2] Institute of Crop Science, Biostatistics Unit, University of Hohenheim, 70599 Stuttgart, Germany
[3] Institute of Crop Science and Resource Conservation, Emmy Noether Group Root Functional Biology, University of Bonn, 53113 Bonn, Germany
[4] Institute of Crop Science and Resource Conservation, Crop Bioinformatics, University of Bonn, 53115 Bonn, Germany
[5] Institute of Crop Science and Resource Conservation, Plant Breeding Department, University of Bonn, 53115, Bonn, Germany
[6] Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) Gatersleben, 06466 Seeland, Germany

Classical concepts of heterosis attribute the superiority of $F_1$-hybrids over their homozygous parents to the complementation of unfavorable by beneficial alleles (dominance) or to heterozygote advantage (overdominance). Here we analyzed backcross hybrids of maize recombinant inbred lines, with an average heterozygosity of ~50%. This genetic architecture allowed us to study the influence of homozygous and heterozygous genomic regions on gene expression in hybrids. We demonstrated, that up to 29% of the heterotic variance in these hybrids is explained by single parent expression (SPE) complementation. In this mode of expression, consistent with the dominance model, genes are expressed in only one of the parents and in the hybrid. Furthermore, we demonstrated that eQTL regulating SPE genes are predominantly located in heterozygous regions of the genome. Thus, we demonstrated that dominance of SPE genes is important for gene activity, while heterozygosity is instrumental for the regulation of these genes. Finally, we identified an SPE gene that regulates lateral root density in hybrids. Remarkably, the activity of this gene depends on the presence of a Mo17 allele in an eQTL that regulates this gene, supporting the notion that the genetic constitution of distant regulatory elements plays a key role for the activity of heterosis-associated genes. In summary, the prevalence of dominance at the level of gene activity and overdominance at the level of gene regulation reconciles these classical genetic concepts and explains how they could both contribute to heterosis.

Funding acknowledgement: DFG German Research Foundation

# Posters

## P1

### An ancient origin of the naked grains of maize

(submitted by Jeffrey Ross-Ibarra <rossibarra@ucdavis.edu>)
Full Author List: Fairbanks, Regina[1]; Ross-ibarra, Jeffrey[1]
[1] University of California, Davis, CA 95616

Adaptation to novel environments requires genetic variation, which may either predate the novel environment or arise as new mutations. The relative importance of standing genetic variation vs. de novo mutations in adaptation remains a fundamental question in evolutionary biology. Selection during domestication has been long used as a model to understand evolutionary processes, providing information not only on the phenotypes selected but also, in many cases, an understanding of the causal loci. Of the multiple causal loci that have been identified in maize, the selected allele can be found segregating in natural populations, consistent with their origin as standing genetic variation. The sole exception to this pattern is the well-characterized domestication locus tga1, which has long been thought to be an example of selection on a de novo mutation. Here, we use a large dataset of maize and teosinte genomes to reconstruct the origin and evolutionary history of tga1. We first estimated the age of tga1-maize using a genealogy-based method, finding that the allele arose approximately 41,000-49,000 years ago, predating the beginning of maize domestication. We also identify, for the first time, tga1-maize in teosinte populations, indicating the allele can survive in the wild. Finally, we compare observed patterns of haplotype structure and mutational age distributions near tga1 with simulations, finding that patterns near tga1 in maize better resemble those generated under simulated selective sweeps on standing variation. These multiple lines of evidence suggest that maize domestication likely drew upon standing genetic variation at tga1 and cement the importance of standing variation in driving adaptation during domestication.

Funding acknowledgement: National Science Foundation (NSF)

## P2

### Analysis of *Ga2* genome structure and activity reveals widespread distribution of functional alleles in modern maize germplasm

(submitted by Adrienne Moran Lauter <adrienne.moranlauter@usda.gov>)
Full Author List: Moran Lauter, Adrienne N[1]; Holland, James B[2]; Scott, M. Paul[1]
[1] USDA-ARS, Corn Insects and Crop Genetics Research Unit, Ames, Iowa, 50011
[2] USDA-ARS, Plant Science Research Unit, Raleigh, North Carolina, 27695

The *Ga2* locus controls unilateral cross incompatibility in *Zea* species. Molecular characterization of this locus indicates that fully functional loci contain two components that function in male and female reproductive tissues, *Ga2P* and *Ga2F* respectively. Some *Zea mays* varieties have been reported to be capable of overcoming the *Ga2* reproductive barrier including the widely used inbred line Mo17. Our objective was to better understand the structure, function and distribution of the *Ga2* locus in *Zea* species. To do this, we first examined the ability to overcome the *Ga2* reproductive barrier in all the NAM parent lines. We also tested several lines derived from Mo17 and found that more than half had some *Ga2m* function. The ability of Mo17 to overcome the *Ga2* reproductive barrier was mapped to the *Ga2* locus. We next examined the structure of the *Ga2* locus. Like the *Ga1* locus, the *Ga2* locus in *Zea mays sp.* contains multiple repeated functional genes and pseudogenes encoding the *Ga2P* pectin methylesterase enzyme. We observed *Ga2F* pseudogenes in the lines, but no functional copies. The organization of these repeat units is similar in the three sweet corn lines examined, and all can overcome the reproductive barrier. The non-sweet corn lines have a variety of genome structures, but these structures do not correlate well with the ability to overcome the *Ga2* reproductive barrier. Levels of transcripts derived from *Ga2P* correlates with the ability to overcome the reproductive barrier in sweet corn, but not in other lines tested. The role of *Ga2P* in *Zea mays* in the apparent absence of a functional female factor remains unclear and may be evidence for an additional role in reproductive tissues.

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

## P3

# Comparative genomic analysis of maize and its wild relatives to identify loci underlying cold tolerance and nitrogen recycling

(submitted by Gretta Buttelmann <glb28@iastate.edu>)
Full Author List: Buttelmann, Gretta L.[1]; Hufford, Matthew B.[1]
[1] Iowa State University, Ames, Iowa, USA 50011

Maize is a warm-season crop, so its growth cycle misses the peak availability of soil nitrogen in the early spring of the Midwestern United States. This mismatch exacerbates leaching of nitrate into waterways and release of nitrous oxide, a potent greenhouse gas, into the atmosphere. A more sustainable system of maize agriculture requires introduction of traits that allow maize to be planted earlier to tap into peak soil nitrogen availability as well as to recycle nitrogen back underground at the end of the growing season for use by the next crop. The Circular Economy that Reimagines Corn Agriculture (CERCA) project, comprised of a team of over 20 research groups, aims to produce an ideotype of maize that exhibits seven key traits associated with cold tolerance and nitrogen recycling. To identify loci associated with nitrogen recycling of the desired ideotype, the Hufford Lab will use comparative genomic approaches to analyze multiple perennial to annual transitions across the Andropogoneae tribe of grasses. Since these transitions likely mark loss of key nitrogen recycling traits, comparing multiple pairs of closely related perennial and annual species can identify contributing loci. For loci contributing to cold tolerance, we will utilize population genetic approaches to analyze cold-tolerant species and populations in the sister genera *Tripsacum* and *Zea*. Here, we describe how comparative genomics can be used to study evolutionary transitions relevant to the CERCA maize ideotype and present the current status of data generation and experimental design.

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

**P4** @mcstitzer

## Comparative grass genomics reveals explosive genome evolution in maize and its wild relatives

(submitted by Michelle Stitzer <mcs368@cornell.edu>)

Full Author List: Stitzer, Michelle C.[1]; Seetharam, Arun S.[2]; Scheben, Armin[3]; Hsu, Sheng-Kai[1]; Schulz, Aimee J.[1]; AuBuchon-Elder, Taylor M.[4]; El-Walid, Mohamed[1]; Ferebee, Taylor H.[1]; Hale, Charles O.[1]; La, Thuy[1]; Liu, Zong-Yan[1]; McMorrow, Sarah[1]; Minx, Patrick[4]; Phillips, Alyssa R.[5]; Syring, Michael L.[2]; Wrightsman, Travis[1]; Zhai, Jingjing[1]; PanAndropogoneae Germplasm, Collaborators[6]; Other PanAndropogoneae, Collaborators[7]; Siepel, Adam[3]; Ross-Ibarra, Jeffrey[5]; Romay, M. Cinta[1]; Kellogg, Elizabeth A.[4]; Buckler, Edward S.[1 8]; Hufford, Matthew B.[2]

[1] Cornell University; Ithaca, NY, USA 14853
[2] Iowa State University, Ames, IA, USA 50011
[3] Simons Center for Quantitative Biology, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, USA 11724
[4] Donald Danforth Plant Science Center, Saint Louis, MO, USA 63132
[5] University of California, Davis, Davis, CA, USA 95616
[6] U. Montpellier; Principa College; U. Georgia; NSF; Mahidol U.; Indiana U.; South China Agricultural U.
[7] Corteva Agriscience, U. Missouri, MaizeGDB
[8] USDA-ARS, Ithaca, NY, USA 14853

Over the last 20 million years, the Andropogoneae tribe of grasses has evolved to dominate 17% of global land area. Domestication of these grasses in the last 10,000 years has yielded our most productive crops, including maize, sugarcane, and sorghum. The majority of Andropogoneae species, including maize, show a history of polyploidy – a condition that, while offering the evolutionary advantage of multiple gene copies, poses challenges to basic cellular processes, gene expression, and epigenetic regulation. To date, understanding the genomic consequences of polyploidy has been limited by the sparse sampling of groups of taxa with multiple polyploidy events. Here, we present 33 genome assemblies from 27 species, including chromosome-scale assemblies of all diploid *Zea* species and subspecies, and two chromosome-scale assemblies from the sister genus *Tripsacum*. These genomes capture 14 independent polyploid formation events, including the shared whole genome duplication between *Zea* and *Tripsacum*. In maize, the after-effects of polyploidy have been widely studied, showing reduced chromosome number, biased fractionation of duplicate genes, and transposable element (TE) expansions. While we observe these patterns within the genus *Zea*, 12 other polyploidy events deviate significantly. Those tetraploids and hexaploids retain elevated chromosome number, maintain nearly complete complements of duplicate genes, and have only stochastic TE amplifications. We hypothesize these contrasting patterns arise from differences in the evolutionary role of polyploidy. In most taxa, polyploidy may buffer genetic load, whereas in maize, it likely fixes heterozygosity from divergent allopolyploid parents. In total, these genomes provide a powerful backdrop for maize geneticists to better understand maize diversity and the evolutionary context of maize genes and alleles.

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

## P5

## Determination of genetic and epigenetic regulations of meiotic recombination during domestication in maize

(submitted by Akwasi Yeboah <akwasiyeboah@ufl.edu>)
Full Author List: Yeboah, Akwasi[1]; Lee, Gwonjin[2]; Flint-Garcia, Sherry A.[3]; Zhao, Meixia[1]
[1] University of Florida, Gainesville, FL 32603
[2] West Virginia State University, Institute, WV 25112
[3] University of Missouri, Columbia, MO 65211

Meiotic recombination involves the exchange of genetic material between homologous chromosomes, playing a key role in evolution and genetic diversity. Meiotic crossovers (COs) are not evenly distributed on chromosomes; instead, they are enriched in hotspots controlled by genetic and epigenetic factors. In maize, meiotic recombination has significantly contributed to maize domestication. However, the specific mechanisms underlying this process remain largely unexplored. This study aims to elucidate the genetic mechanisms driving meiotic recombination during maize domestication and to compare how meiotic recombination differs between sexes. Our investigation encompasses a diverse set of maize lines, including 5 teosintes, 7 landraces, and 15 cultivars. These lines were selected because they maximize genetic diversity and thus are most likely to show variation in recombination. Our results reveal that the teosinte line BK4 and the landrace MR19 exhibited longer genetic distances and higher crossover numbers, whereas BK1 and MR11 showed the lowest, compared to the other teosinte and landrace lines. Of the 27 total lines including cultivars, BK4 and MR19 were still among the highest in crossover numbers, while BK1 and CML103 showed the lowest. CO hotspot analysis revealed that most hotspots were located at the distal ends of each chromosome across all lines. Additionally, our results reveal significant differences in CO number and recombination rate between sexes, and these differences were dependent on different maize genetic backgrounds. Furthermore, analysis of distance CoC showed that teosinte lines exhibited higher, though not statistically significant, levels of CO interference compared to other maize lines. In our ongoing research, we are collecting immature anthers from maize plants exhibiting extreme CO numbers and recombination rates for transcriptome and DNA methylation analysis. This work aims to uncover the genetic and epigenetic factors underlying meiotic recombination in maize, thereby enhancing our understanding of this crucial biological process.

Funding acknowledgement: National Science Foundation (NSF)

## P6

## Discovering mechanisms of maize abnormal chromosome 10 (Ab10) meiotic drive through comparative genomics

(submitted by Mingyu Wang <mw36149@uga.edu>)
Full Author List: Wang, Mingyu[1]; Gent, Jonathan[2]; Dawe, R. Kelly[3]
[1] Institute of Bioinformatics, University of Georgia, Athens, GA 30602
[2] Dept of Plant Biology, University of Georgia, Athens, GA 30602
[3] Dept of Genetics, University of Georgia, Athens, GA 30602

Maize abnormal chromosome 10 (Ab10) encodes a meiotic drive system that activates two types of heterochromatic knobs, converting them into neocentromeres that cause transmission ratio distortion. Ab10 encodes two neocentromere-activating kinesins: *KINESIN DRIVER* (*KINDR*) activates knob180 knobs, and *TR-1 KINESIN* (*TRKIN*) activates TR-1 knobs. However, other important elements involved in the meiotic drive system are still unclear. In this study, we performed a comparative genomic analysis of seven Ab10 haplotypes. We found several candidate genes potentially associated with meiotic drive and identified shared structural features among the Ab10 groups. Moreover, we estimated the phylogenetic relationships among these haplotypes, particularly of their *kindr* and *trkin* genes. These results will provide insights into the mechanisms of Ab10 meiotic drive and its global effects on knob-linked allele frequencies.

Funding acknowledgement: National Science Foundation (NSF)

## P7

### Does maize diversity mirror human diversity across the Americas?

(submitted by Samantha Snodgrass <ssnodgrass@ucdavis.edu>)
Full Author List: Snodgrass, Samantha[1]; Gerardo Medina, Santiago[2]; Moreno Estrada, Andrés[2]; Coop, Graham[1 3]; Ross-Ibarra, Jeffrey[1 3]
[1] Evolution and Ecology, UC Davis, Davis, CA, US
[2] Centro de Investigación y Estudios Avanzados del IPN (Cinvestav), Mexico
[3] Center for Population Biology, UC Davis, Davis, CA, US

Maize depends completely on human intervention for survival and reproduction. While maize pollen can travel short distances by wind, maize kernels can only move into new areas by human cultivation and are otherwise at high risk of seed predation. Likewise, different human communities may cultivate and select maize differently. This tight dependence of maize and different selective regimes can be modeled as bio-cultural coevolution. Under this framework, the spatial-genetic relationships between maize populations should mirror the spatial-genetic relationships between indigenous human communities. We test this hypothesis using the Seeds of Discovery maize dataset, which includes GBS sequencing of thousands of traditional maize varieties from Mexico and South America, and the Mexican Biobank human dataset, which includes whole genome sequences of hundreds of individuals with indigenous ancestry from Mexico and South America. We measure the correlation of genetic relatedness with spatial position for each species. For maize, we additionally remove ancestry from the wild relative Zea mays ssp. mexicana to account for the strong effects of mexicana introgression along the elevation gradient in the Mexican highlands. Then we group maize samples with human samples to measure the correlation of the spatial-genetic relatedness between species. In preliminary results, humans have stronger spatial-genetic structure than maize, but both show significant signals of spatial-genetic structure. This work provides insight into the impact of trade between and migration of humans on diversity patterns of maize.

Funding acknowledgement: National Science Foundation (NSF)

## P8

### Evolution of the protein complex involved in early recombination in plants

(submitted by Arnaud Ronceret <arnaud.ronceret@ibt.unam.mx>)
Full Author List: Rafiei, Nahid[1]; Palacios-Martínez, Janet[1]; Rueda, Patricia[1]; Ronceret, Arnaud[1]
[1] Department of Plant Molecular Biology, Instituto de Biotecnología (IBT), Universidad Nacional Autónoma de México (UNAM), Cuernavaca, Morelos, México CP62110

During meiosis, replicated chromosomes are reshuffled at various crossing-over (CO) sites. The creation of stochastic CO starts with the formation of stochastic DNA double strand breaks (DSBs) during early prophase I [1,2]. DNA DSBs are created by the enzymatic activity of a topoisomerase-like complex containing SPO11 known as the early recombinosome [1-5]. In plants, two SPO11 proteins SPO11-1 and SPO11-2 form a heterodimer also associate with additional factors called MTOPVIB, PUTATIVE RECOMBINATION DEFECT 1 (PRD1), PRD2, PRD3, DSB FORMING (DFO) [5]. All these factors are individually required for the formation of DSB formation in Arabidopsis and rice. Some of them are demonstrated to be also required in maize, barley and wheat [1,3,4]. The SPO11 complex was described to form three sub-complexes [5]. The core complex contains the SPO11-1/SPO11-2/MTOPVIB that conserve the conformation of a topoisomerase type VI [5]. The PRD1 protein links the different subcomplexes together. PHS1, PRD2, DFO, PRD3 can contain unstructured domains. We analyze the evolution of conservation and divergence of these proteins in plants. The core complex is more conserved than the additional subcomplexes and some protein of the complex such as PHS1 have divergent requirement for DSB formation in Arabidopsis and maize [5]. Several of these proteins contain unstructured regions. We explore the modeling of early recombinosome complex through Alfafold3. The early recombinosome can form a topoisomerase-like structure in accordance with previous two by two interactions analysis. By comparing the organization of both Arabidopsis and maize early recombinosome complexes, we identify possible differences between these divergent early recombinosome complexes in their association with two or with only one DNA molecules. We will also discuss the hypothetical implications that the established topoisomerase-like nature of the SPO11 complex can have in creating DSB in only one of the two replicated chromatids of the early prophase I meiotic chromosomes.

Gene / Gene Models described: *SPO11-1, SPO11-2, MTOPVIB, PRD1, PRD2, PRD3, PHS1, DFO*; Zm00001eb214790, Zm00001eb172550, Zm00001eb227420, Zm00001eb389920, Zm00001eb180360, Zm00001eb404350, Zm00001eb382430, Zm00001eb195020
Funding acknowledgement: CONAHCYT, UNAM

## P9

## Evolutionary determinants of gene expression in maize

(submitted by Amy Pollpeter <amyrp@iastate.edu>)
Full Author List: Pollpeter, Amy R.[1]; Gilbert, Amanda M.[2]; Hirsch, Candice N.[2]; Severin, Andrew J.[1]; Hufford, Matthew B.[1]
[1] Iowa State University, Ames, Iowa, USA 50011
[2] University of Minnesota, St. Paul, Minnesota, USA 55108

The maize Nested Association Mapping (NAM) population has significantly contributed to our understanding of maize genetics over the past decade, offering numerous insights into the natural variation of complex traits across diverse environments. Characterization of the 26 NAM founder lines has enabled detailed exploration of variation at genetic sequence, methylation, and expression levels. Here, we utilize expression data from ten plant tissues in the NAM founders to evaluate how the frequency of genes in modern maize, the evolutionary age of genes, and a gene's role in temperate adaptation are linked to patterns of gene expression. We evaluate three main hypotheses: First, we examine whether measures of tissue specificity, such as Tau and the Coefficient of Variation (COV), differ across pan-genes classified as core, near-core, dispensable, or private. We predict that core and near-core pan-genes will exhibit lower tissue specificity compared to dispensable and private genes. Second, we determine whether the age of a gene, as determined by a phylostrata analysis, explains patterns of expression. We hypothesize that older genes will be more highly expressed and less tissue-specific in their expression. Third, we investigate gene expression differences between NAM founder lines adapted to tropical versus temperate environments. We hypothesize that differentially expressed genes between these populations will show higher tissue specificity and substantial rewiring of expression. These results not only enhance our fundamental understanding of maize adaptation but also have practical implications for improving crop resilience and expanding agricultural productivity in diverse and changing environments.

## P10

## Extensive modulation of a conserved cis-regulatory code across 625 grass species

(submitted by Charles Hale <coh22@cornell.edu>)
Full Author List: Hale, Charles O[1]; Hsu, Sheng-Kai[2]; Zhai, Jingjing[2]; Schulz, Aimee J[1]; AuBuchon-Elder, Taylor[3]; Costa-Neto, Germano[2]; Hufford, Matthew B[4]; Kellogg, Elizabeth A[3]; La, Thuy[2]; Marand, Alexandre P[5]; Romay, M Cinta[2]; Seetharam, Arun[3]; Stitzer, Michelle[2]; Buckler, Edward S[1 2 6]
[1] Section of Plant Breeding and Genetics, Cornell University, Ithaca, NY USA 14853
[2] Institute for Genomic Diversity, Cornell University, Ithaca, NY USA 14853
[3] Donald Danforth Plant Science Center, St. Louis, MO USA 63132
[4] Iowa State University, Ames, IA USA 50011
[5] University of Michigan, Ann Arbor, MI USA 48109
[6] USDA-ARS; Ithaca, NY, USA 14853

The growing availability of genomes from non-model organisms offers unprecedented opportunities to use comparative genomics to pinpoint functional loci underlying trait variation. Cis-regulatory regions drive much of phenotypic evolution, but linking these sequences to specific functions remains a major challenge. We identified a set of 496 cis-regulatory sequence motifs enriched in the regulatory regions of diverse grass species. 82% of motifs were consistently enriched across all species, suggesting the presence of a deeply conserved regulatory code. Having established a conserved set of motif sequences across grasses, we then quantified conservation of specific motif instances across 625 grass species. We uncovered widespread gain and loss of cis-regulatory motifs across species, with a nonlinear decay in motif conservation over increasing evolutionary time scales. Approximately 60% of maize motif instances were found to be conserved in sorghum, and 50% in rice. Motif conservation varied widely across genes. We observed subtly higher motif conservation at transcription factor genes compared to downstream target genes, suggesting that the regulatory regions of highly pleiotropic genes may be under stronger constraint. We then tested for adaptive cis-regulatory changes, using phylogenetic mixed models to detect motif gains and losses associated with environmental niche transitions. Our results revealed polygenic patterns of adaptation, with weak but significant convergence at several hundred individual motif instances. These findings support a model in which cis-regulatory evolution occurs primarily via extensive turnover of a conserved regulatory code. Regulatory changes at hundreds if not thousands of genes appear to underpin environmental adaptation. Our findings underscore the potential of comparative genomics and phylogenetic mixed models to discover genetic loci underlying trait variation.

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

## P11 @LinaLpzc

## Genetic and phenotypic characterization of wild teosinte alleles introgressed into elite maize line CML311

(submitted by Lina Lopez-Corona <llopezc@ncsu.edu>)
Full Author List: Lopez-Corona, Lina[1 2 6]; Guerrero-Corona, Abraham[4]; Samayoa-Lopez, L Fernando[5]; De La Cruz-Larios, Lino[3]; Rellan-Alvarez, Ruben[2]; Sanchez-Gonzalez, J Jesus[3]; Holland, James[1 2]
[1] U.S. Department of Agriculture, Agricultural Reserach Servise (USDA-ARS) 3127 Ligon St, Raleigh, NC, United States, 27606
[2] North Carolina State University, Raleigh, North Carolina, United States, 27695
[3] Universidad de Guadalajara, Centro Universitario de Ciencias Biológicas y Agropecuarias (CUCBA), Cam. Ramón Padilla Sánchez 2100, Las Agujas, Zapopan, Jal. Mexico, 44600
[4] Salk Institute for Biological Studies, 10010 N Torrey Pines Rd, La Jolla, California, United States, 92037
[5] INARI, 1281 Win Hentschel Blvd w1108, West Lafayette, Indiana, United States, 47906
[6] Oak Ridge Institute for Science and Education (USDA-ARS), 1299 Bethel Valley Rd, Oak Ridge, TN, USA, 37830

Modern maize (Zea mays L. ssp. mays) is the result of domestication from wild relatives (Zea spp.) and subsequent breeding, which in the last century targeted mostly yield under optimal conditions. Alleles from wild relatives lost from modern maize may contribute other beneficial traits or better adaptation to lower input conditions. To study the potential of wild relative's alleles to contribute to modern maize improvement, we generated 2208 Near-isogenic lines (NILs) were derived from crosses between 85 teosinte plants from 78 wild populations (representing almost all known Zea taxa) as donor male parents with the elite tropical line CML311 as recurrent parent. Introgressions were mapped using reduced representation sequencing and a hidden Markov model. The lines were phenotypically evaluated in three environments in Mexico. A subset of 3114 SNP markers was selected to represent the introgressed segments for QTL mapping. Here we evaluated the consistency of QTL effects due to introgressions from different teosinte donors. This set of NILs represents a wide diversity of wild alleles introgressed into a modern tropical inbred line with good adaptation to tropical environments.

Funding acknowledgement: United States Department of Agriculture (USDA)

## P12

## Genetic legacies of the first ancient South American state in archaeological maize

(submitted by Heather Chamberlain <heather.chamberlain.irwin@univie.ac.at>)
Full Author List: Chamberlain, Heather N.[1 2]; Pinhasi, Ron[1]; Somerville, Andrew D.[2]
[1] University of Vienna, Vienna, Austria
[2] Iowa State University, Ames, IA, USA, 50010

Moquegua Valley in South Peru has an ancient legacy of maize cultivation dating back long before European contact. When and in what form maize arrived in South Peru is still a mystery. While maize arrived in Peru ~6,600 BP at least partially domesticated, documented movement of maize within the Andes and admixture with Mexican varieties suggests a complicated evolutionary history. Over thousands of years, the people of Moquegua Valley gradually increased maize cultivation, with more diverse subsistence farming dominating for much of the region's history. Trade networks were well developed between the peoples of the Valley and the high-altitude regions of the Andes, including the Tiwanaku state. This research explores the co-evolution of humans and maize by analyzing genetic changes in maize over 1,500 years of human occupation in the Moquegua Valley. By examining highland and lowland maize alleles and tracking traits under selection, we assess how the expansion of the Tiwanaku state influenced maize genomics. Using AMS-dated archaeological maize samples, we map the genetic changes in maize from ancient times to better understand the impact of Tiwanaku colonization on local maize varieties. Comparisons to modern maize help assess the legacy of these processes and put ancient specimens in context. Moreover, broader impacts will empower local farmers through documentation and curation of diverse regional germplasm, provide educational opportunities through training of undergraduate and graduate students, and establish a meaningful link between the past and the present for future researchers.

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## P13

### Resolving maize domestication and subpopulation divergence using long terminal repeat retrotransposons

(submitted by Christopher Benson <benson.140@osu.edu>)
Full Author List: Benson, Christopher[1]; Ou, Shujun[1]
[1] Ohio State University; Dept. Molecular Genetics; Columbus, Ohio, 43202

Maize is morphologically diverse, with subpopulations adapted to unique agricultural and ecological niches. The admixed domestication histories of maize subpopulations from teosinte are challenging to resolve, primarily due to shallow evolutionary timescales and the limitations of SNP-based methods, including ascertainment bias of genotype calling, prevalence of incomplete lineage sorting (ILS), introgression, and rapid divergence of subpopulations. Long terminal repeat retrotransposons (LTR-RTs) are transposable elements that are identical upon insertion. Mutations to LTR-RTs reflect their insertion time, enabling fine-grained calculations of genetic distances at shallow timescales and providing valuable phylogenetic signals for studying molecular evolution. In this study, we compare the insertion times of shared and unique LTR-RTs in 38 maize genome assemblies to explore maize domestication at the subpopulation level. We use 2.2 million LTR retrotransposons spanning 22.4 Gb, 26% of all genomic sequences, to resolve genetic distance across chromosomes. Our whole-genome analysis suggests that sweet corn and flint corn are closely related to each other and diverged from the common ancestor connecting them to tropical and temperate maize at a genetic distance of 0.56%, while tropical and temperate maize have a genetic distance of 0.09% from their common ancestor. Analysis of shared and unique LTR-RTs across chromosomes suggests that sweet and flint corn received novel gene flow from teosinte when compared to tropical and temperate maize, which likely facilitated phenotypic novelty and local adaptations. This study provides novel insights into the domestication of modern maize and highlights the power of leveraging presence-absence variation between self-dated LTR-RTs to infer genetic distances.

Funding acknowledgement: United States Department of Agriculture (USDA)

## P14 @subhashmvs

### Revealing the genetic architecture for different nitrogen responses in sorghum

(submitted by Sai subhash Mahamkali VS <mahankalisubhash03@gmail.com>)
Full Author List: M.V.S, Sai Subhash[1 2]; Jin, Hongyu[1 2]; Xu, Gen[1 2]; Turkus, Jon[1 2]; Ge, Yufeng[2 3]; Schnable, James[1 2]; Wang, Zhen[1 2]; Yang, Jinliang[1 2]
[1] Department of Agronomy and Horticulture, University of Nebraska-Lincoln, Lincoln, N 68583 USA
[2] Center for Plant Science Innovation, University of Nebraska-Lincoln, Lincoln, NE 68583 USA
[3] Department of Biological Systems Engineering, University of Nebraska-Lincoln, Lincoln, NE 68583, USA

Nitrogen (N) is an essential macronutrient, often supplied as synthetic fertilizer for enhancing grain yield. It is also the most expensive and energy intensive inputs in global agricultural production. Most cereal crops are inefficient in N usage, leading to a series of environmental and economic concerns. Therefore, increasing N use efficiency (NUE) in agriculture production is critical for environmental sustainability and food security. Sorghum is the fifth most cultivated cereal and considered as a multipurpose commodity crop. Despite being a C4 crop, to achieve a high photosynthetic capacity for higher yields, sorghum need to be supplied with high quantity of inorganic N fertilizer. In this study, we collected data under different N conditions from both greenhouse and field using 346 accessions from the sorghum association panel (SAP). After phenotypic data analysis, we observed large variations for different traits under high N and low N conditions. Subsequently, using publicly available whole-genome sequencing (WGS) data, we employed a variant calling pipeline with the latest sorghum reference genome. Following data quality checking and filtration, we retained a total of 4.7 million SNPs and 6.6 million indels. After conducting GWAS using the filtered dataset, we identified a number of trait-associated loci for traits under different N conditions and N-responsive traits. Overall, this study revealed the polygenic nature of the N responsive traits measured in both greenhouse and field conditions and provided candidate genes for NUE improvement in sorghum.

Funding acknowledgement: Department of Energy (DOE)

## P15

### Sex-specific patterns of meiotic recombination are determined by maize lines from different climate zones.

(submitted by Gwonjin Lee <gwonjin.lee@wvstateu.edu>)
Full Author List: Lee, Gwonjin[1 2]; Zhao, Meixia[2]
[1] Department of Biology, West Virginia State University, Institute, WV 25064
[2] Department of Microbiology and Cell Science, University of Florida, Gainesville, FL 32611

Meiotic recombination, occurring during meiosis, is a fundamental biological process involved in crossovers (COs), which affect genetic diversity in offspring. Although some mechanisms of COs in plants have been characterized, a comprehensive understanding of the patterns and regulation of COs, especially in female and male meiocytes, remains to be elucidated. In this study, we used parental inbred lines from the maize nested association mapping (NAM) populations, representing more than 85% of intraspecies genetic diversity in maize, to explore variations and factors involved in meiotic recombination between the sexes. Through analyses of CO landscapes based on various sequencing data, we found that the pattern of meiotic recombination differs considerably between tropical and temperate lines in males, while remaining constant in females. Our transcriptomic profiling indicated that various heat shock proteins (HSPs) are up-regulated during male meiosis in the temperate line, whereas they are down-regulated in the tropical line. Furthermore, CO frequencies in females were negatively correlated with CHH (where H = A, T, or C) methylation levels in somatic cells, indicating that standing variation in CHH methylation could impact meiotic recombination variability in female meiocytes across different lines. These observations, together with further investigations into genetic and epigenetic factors, may contribute to a comprehensive understanding of the regulation of sex-specific meiotic recombination, differing by genetic background and origin of cultivars.

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## P16 @ManishaMuna

### Structural variation has a limited role in influencing genome-wide differential gene expression patterns in maize

(submitted by Manisha Munasinghe <mmunasin@umn.edu>)
Full Author List: Munasinghe, Manisha A.[1 2]; Read, Andrew[3]; Springer, Nathan M.[1]; Brandvain, Yaniv[1 4]; Hirsch, Candice N.[2]
[1] Department of Plant and Microbial Biology, University of Minnesota, St. Paul, MN, 55108, USA
[2] Department of Agronomy and Plant Genetics, University of Minnesota, St. Paul, MN, 55108, USA
[3] USDA-ARS, Plant Science Research Unit, St. Paul, MN, 55108, USA
[4] Department of Ecology, Evolution and Behavior, University of Minnesota, St. Paul, MN, 55108, USA

Structural variants refer to large (> 50 bp) insertions or deletions of DNA sequences. While there are often fewer structural variants (SVs) within a genome in comparison to the number of single nucleotide polymorphisms, SVs routinely result in more nucleotide changes between genomes. However, the large size and strong association between SVs and repetitive sequences have historically made it difficult to identify them. In spite of this, there are a number of examples across major crop species of genomic structural variation impacting agronomic traits. Much of this work has been limited to single-locus explorations, and it remains unclear whether these observations are a rarity or widespread across the genome. Here, we use the NAM population to explore whether structural variation in the promoter region of a gene results in differential expression. We leverage publicly available data to extract the location of structural variants across these lines, ascertain how much of that structural variant derives from transposable element sequence, and test whether this variation leads to differential expression across tissues and between lines. While we find thousands of genes that result in differential expression, we find very few commonalities or patterns amongst our significant hits. Many of these genes appear to be of limited function as well. Furthermore, when we randomly downsample the number of included tissues and retest for differential expression, we find that many significant genes become nonsignificant, indicating a tissue specific nature to the effect of the SV. Our results suggest that significant differential expression between structural haplotypes may depend on very specific circumstances and that the majority of differentially expressed genes are present only because the variation in gene expression has minimal phenotypic impact.

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## P17

### The effect of modern breeding on rhizosphere microbiome under different nitrogen conditions in maize

(submitted by Yuchu Ma <nathanchu@huskers.unl.edu>)
Full Author List: Ma, Yuchu[1]; Xu, Gen[1]; Hao, Jingjie[1 2]; P. Schachtman, Daniel[1 2]; Yang, Jinliang[1]
[1] Department of Agriculture and Horticulture, University of Nebraska-Lincoln, Lincoln, NE 68588
[2] Center for plant science innovation, University of Nebraska-Lincoln, Lincoln, NE 68588

The symbiotic relationship between plant host and rhizosphere microbiome (rhizobiome) plays an important role in enhancing plant nutrient acquisition, disease control, and the host plant's resistance to environmental stress. Our previous studies using historical inbreds suggest that both host genetics and host root-associated microbiomes have been reshaped during modern breeding. To harness the partnership between host genetics and natural occurring beneficial microbes, in this study, we investigate the rhizobiome using a set of under utilized tropical material that backcrossed to the temperate background, or Backcrossed Germplasm Enhancement of Maize (BGEM) population. In the field experiment, we planted the BGEM population (300 inbreds and 200 hybrids) according to an incomplete block design with two nitrogen treatments (high N and low N) , each with two replications, over three consecutive years. In 2022, we excavated the roots and profiled the rhizobiome using 16S rRNA sequencing. Analyzing the phenotypic data revealed the aboveground and belowground traits exhibited varying levels of heterosis under high N and low N conditions. Interestingly, among the 2,168 amplicon sequence variants (ASVs) identified in BGEM population, a number of them exhibited higher abundance in hybrids than their respective inbred parents. Our study reveals the effects of the modern breeding in reshaping the belowground root and rhizobiome characteristics, and sheds light on future microbiome-enabled plant improvement.

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## P18

### The genetic basis of environmental adaptation in grasses

(submitted by Sheng-Kai Hsu <sh2246@cornell.edu>)
Full Author List: Hsu, Sheng-Kai[1]; Schulz, Aimee J[2]; Hale, Charles O[2]; Costa-Neto, Germano[1]; Stitzer, Michelle C[1]; Miller, Zhachary R[1]; Wrightman, Travis[2]; AuBuchon-Elder, Taylor[3]; Kellogg, Elizabeth A[3 4]; Romay, M Cinta[1]; Buckler, Edward S[1 2 5]
[1] Institute for Genomic Diversity, Cornell University, Ithaca, NY, USA 14853
[2] Section of Plant Breeding and Genetics, Cornell University, Ithaca, NY USA 14853
[3] Donald Danforth Plant Science Center, St. Louis, MO, USA 63132
[4] Arnold Arboretum of Harvard University, Boston, MA, USA 02130
[5] USDA-ARS, Robert W. Holley Center for Agriculture and Health, Ithaca, NY, USA 14853

Grasses (Poaceae), encompassing major cereal crops such as wheat, rice, and corn, are foundational to global food security. This family is one of the largest and most important plant families, containing around 780 genera and 12,000 species distributed globally. Their presence spans from arctic tundras to tropical forests, covering approximately 40% of the Earth's land surface, excluding Greenland and Antarctica. Understanding the genetic mechanisms that underpin this adaptability is crucial for enhancing agricultural resilience against climate change. In this study, we investigate the genetic basis of environmental adaptation in over 500 grass species by integrating genomic and biogeographic data. We employ cross-species association approaches to elucidate the genetic variation associated with adaptation to key environmental factors like precipitation and temperature. Our analysis aims to identify specific genes and genetic pathways that confer tolerance to these stressors, potentially uncovering overlap in the genetic mechanisms of multiple stress resistance that have evolved in these species. By delineating these genetic factors, our research contributes to the strategic development of crop varieties better suited to the changing climate, thereby enhancing food security.

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

## P19

### The influence of heavy metal stress 1 on the evolutionary transition of teosinte to maize

(submitted by Jonathan Acosta <jonathan.acosta@cinvestav.mx>)
Full Author List: Acosta-Bayona, Jonathan J.[1]; Vallebueno-Estrada, Miguel[2]; Vielle-Calzada, Jean-Philippe[1]
[1] Unidad de Genómica Avanzada 8 Langebio, Centro de Investigación y de Estudios Avanzados del IPN, Km 9.6 9 Libramiento Norte Carretera Irapuato-León, Irapuato Guanajuato, México, CP36825
[2] 2Swedish University of Agricultural Sciences, Umeå, Sweden

Maize originated from teosinte parviglumis following a subspeciation event that occurred in volcanic regions of Mesoamerica about 9,000 years ago; however, the extent to which environmental factors influenced this evolutionary transition remains uncertain. We explored the impact of heavy metal (HM) stress by exposing both subspecies to sublethal concentrations of copper and cadmium. We also assessed the genetic diversity of loci encompassing three genes affected by domestication and encoding heavy metal ATPases of the P1b family, or multicopper oxidases. All map in the short arm of chromosome five, in a genomic region containing multiple linked QTLs with pleiotropic effects on domestication. A genomic analysis of the full chromosome shows that loci encompassing these three HM response genes show strong positive selection as compared to previously identified domestication genes. We determined their developmental expression pattern and conducted a phenotypic analysis of their mutants. Exposure of teosinte parviglumis to HM stress results in plant architecture reminiscent of extant maize, and upregulation of Teosinte branched1 (Tb1) in the meristem. Their mutant phenotypes indicate that they are mainly involved in optimizing the number of female inflorescences and seminal roots, restricting plant height under HM stress. Our results suggest that abiotic stress influenced the evolutionary transition that gave rise to extant maize through the activity of heavy metal response genes and their phenotypic effects

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## P20

### The molecular evolution of perenniality across the grasses

(submitted by Aimee Schulz <schu4332@umn.edu>)
Full Author List: Schulz, Aimee J[1]; AuBuchon-Elder, Taylor[2]; Costa Neto, Germano[3]; Hale, Charles O[1]; Seetharam, Arun S[4 5]; Stitzer, Michelle C[3]; Romay, M Cinta[3]; Hufford, Matthew B[4]; Kellogg, Elizabeth A[2]; Buckler, Edward S[1 3 6]; Hsu, Sheng-Kai[3]
[1] Section of Plant Breeding and Genetics, Cornell University, Ithaca, NY USA 14853
[2] Donald Danforth Plant Science Center, St. Louis, MO USA 6313
[3] Institute for Genomic Diversity, Cornell University, Ithaca, NY USA 14853
[4] Department of Ecology, Evolution, and Organismal Biology, Iowa State University, Ames, IA USA, 50011
[5] Rosen Center for Advanced Computing, Purdue University, West Lafayette, IN 47907
[6] USDA-ARS; Ithaca, NY, USA 14853

The Poaceae family contains some of the most productive and economically important agricultural crops such as maize, rice, wheat, sorghum, Miscanthus, and sugarcane, and has adapted to a wide range of environments. Notably, while most of the Poaceae are perennial, there have been dozens of transitions to an annual life history. Perennials have multiple traits that can be harnessed to confer an advantage to agricultural crops by reducing their environmental impact, such as nitrogen remobilization and freezing tolerance. Many of these favorable traits are hypothesized to have been lost during the transition to annuality. Through the curation of 172 long read and assembly of 635 short read Poaceae genomes, we directly test the loss of function hypothesis. We find, on average, 151 fewer genes in annual species compared to perennials. Annuals species have a 66% enrichment for more premature stop codons, and additionally show a 9.3-fold enrichment for the loss of nucleotide conservation compared to perennials, highlighting that hundreds of genes may be involved in perennial to annual transitions. Using a phylogenetic mixed model, we show that genes involving meristematic control are more conserved in perennials. When evaluating the presence or absence of rhizomes, we find the contrary, where presence of rhizomes is associated with gene loss of function. Our phylogenetic mixed model indicates that reproductive-related genes are driving part of this loss of function response. The results from this research will provide a launching point for future work to understand the adaptive potential of perennials and develop grass varieties that are more perennial-like and better adapted for climate change.

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

## P21

### A modified replication timing profiling method improves early replication detection and facilitates comparison of multiple genotypes

(submitted by Emily Wheeler <eamarkha@ncsu.edu>)
Full Author List: Wheeler, Emily[1]; Mickelson-Young, Leigh[1]; Wear, Emily[1]; Burrorghs, Mason[1]; Concia, Lorenzo[2]; Bass, Hank[3]; Thompson, William[1]; Hanley-Bowdoin, Linda[1]
[1] Department of Plant and Microbial Biology, North Carolina State University, Raleigh, NC 27595
[2] Texas Advanced Computing Center, Austin, TX, 78758
[3] Florida State University, Tallahassee, FL, 32306

Replication timing (RT), the temporal order in which the genome replicates, is considered a functional outcome of multiple cellular processes and chromatin organization. Two approaches to measure RT are the Repli-seq and DNA copy number (also called S/G1) methods. We previously adapted Repli-seq using 5-ethynyl-2'- deoxyuridine (EdU) pulse-labeling and bivariate flow sorting, followed by immunoprecipitation of EdU labeled DNA. While the Repli-seq approach offers high resolution and exposes heterogeneity in timing, the S/G1 method, which does not require an immunoprecipitation step, is simpler, faster and less resource-intensive. Here, we modified the S/G1 technique by using EdU labeling (EdU-S/G1) to facilitate better separation of replicating from non-replicating nuclei during flow sorting, which enables the collection of a pure G1-phase sample. When comparing the three methods, we found that profiles from the S/G1 and EdU-S/G1 methods are highly correlated with each other and with Repli-seq profiles for early replication. We also found that the EdU-S/G1 approach offers better resolution of replication in early and late S phase than the conventional S/G1 method. Because of the high reproducibility of RT profiles among all three methods, concerns such as cost and sample availability can drive the decision of which method to choose. The low input requirements of the S/G1 methods are especially advantageous when comparing replication time across multiple meristem tissues or genotypes. We chose the more sensitive EdU-S/G1 approach to compare the RTs of homologous genes in Sorghum bicolor and the Zea mays lines B73 and NC350.

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## P22

### Annotating of active LTR retrotransposons in course-based genomics research

(submitted by Mariana Chavez-Carranza <mchavezc03@hotmail.com>)
Full Author List: Chavez-Carranza, Mariana T[1]; Gardner, Patrick[1]; Isles, Taylor[1]; Du, Charles[1]
[1] Department of Biology, Montclair State University, Montclair, NJ 07043, USA

Long terminal repeat (LTR) retrotransposons are a major class of transposable elements that play significant roles in genome evolution, regulation, and structure. These transposable elements are characterized by their flanking LTR sequences, replicated via "copy and paste" mechanism involving RNA intermediates and reverse transcription. In this study, we provide a comprehensive analysis of LTR retrotransposons focusing on their structural diversity, evolutionary dynamics, and functional impacts. Using high-throughput sequencing data and computational annotation pipelines, we identify conserved motifs and lineage-specific expansions, shedding light on their evolutionary history. Although identifying LTRs manually through sequence alignment and motif recognition is theoretically possible, it remains a labor-intensive and error-prone process. Therefore, employing computational tools to efficiently detect LTRs and analyze their features across large genomic datasets. All of this work was produced manually and verified with software to make sure that there is little to no error. The findings highlight the dual nature of LTR retrotransposons are drivers of genomic innovation and potential sources of instability, offering new perspectives on their contributions to genome biology and evolution. An analysis was performed on 72 sequences with supposed active LTR retrotransposons, however, only 2 of the sequences gave us everything we were looking for with minimal mutations.

## P23

## Bacterial strains isolated from *Vellozia* spp. promote maize growth

(submitted by Sylvia Morais de Sousa Tinoco <sylvia.sousa@embrapa.br>)
Full Author List: Ferreira, Nataly F[1]; Souza, Rafaela F A[2]; Maia, Andre L M[2]; Gerhardt, Isabel R[3 4]; Dante, Ricardo A[3 4]; Lana, Ubiraci F P[5]; Tinoco, Sylvia M S[1 3 4 5]
[1] Federal University of São João del-Rei (UFSJ), São João del-Rei, MG, Brazil, 36301-158
[2] University Center of Sete Lagoas (Unifemm), Sete Lagoas, MG, Brazil, 35701-242
[3] Genomics for Climate Change Research Center (GCCRC) Campinas, SP, Brazil, 13083-875
[4] Embrapa Digital Agriculture, Campinas, SP, Brazil, 13083-886
[5] Embrapa Maize and Sorghum, Sete Lagoas, MG, Brazil, 35701-970

The “campo rupestre” (rupestrian grassland), an ecoregion from Brazil with nutritionally poor soils, host the Velloziaceae family, whose species exhibit various morphophysiological strategies for drought resistance, ranging from desiccation tolerance (*Vellozia nivea*) to drought avoidance by remaining evergreen (*Vellozia intermedia*). Investigating the microbiome of these species can unveil microorganisms adapted to the region's extreme conditions, such as bacteria from the genera *Bacillus* and *Paenibacillus*. These bacteria hold potential as bioinoculants due to their ability to form resistant endospores and their diverse plant growth-promoting (PGP) traits. This study focused on identifying and characterizing bacterial strains capable of solubilizing insoluble phosphate sources and tolerating osmotic stress. Strains from the root microbiomes of *V. nivea* and *V. intermedia* were cultivated in LB medium and subjected to heat shock at 80°C, recovering 87 out of 242 strains. These strains were tested for phosphate solubilization in media containing calcium phosphate [$Ca_3(PO_4)_2$] and phytic acid ($C_6H_{18}O_{24}P_6$), as well as for water stress tolerance using media supplemented with sorbitol or NaCl. Among the 87 strains, 37 were capable of solubilizing P from $C_6H_{18}O_{24}P_6$, while 34 efficiently solubilized P from $Ca_3(PO_4)_2$. In the sorbitol tolerance test, 73 strains resisted 405 g $L^{-1}$, 25 tolerated 520 g $L^{-1}$, and 4 withstood 780 g $L^{-1}$. Similarly, 68 and 62 strains tolerated 10% and 20% (w/v) NaCl, respectively. A total of 24 strains were effective in both phosphate solubilization and tolerance to 405 g $L^{-1}$ sorbitol, five strains solubilized insoluble P sources and tolerated up to 520 g $L^{-1}$ sorbitol, and three strains excelled in all tests. Additionally, molecular identification was performed using 16S rDNA sequencing. Following genetic screening, 18 strains of *Bacillus* and *Paenibacillus* were tested on maize seedlings, both with and without Polyethylene Glycol (PEG) 6000, to assess their PGP abilities under controlled conditions. Some strains demonstrated PGP potential under normal conditions, while others exhibited the ability to mitigate drought stress, highlighting their potential as bioinoculants.

Funding acknowledgement: Embrapa, Fapesp, Fapemig, INCT-CNPq, Finep, Capes

# P24

## Building a synthetic cuticle: Characterizing the impact of maize transcription factors on cuticle biosynthetic pathways

(submitted by Madison Lane <mlane@iastate.edu>)
Full Author List: Lane, Madison O[1 2]; Tong, Jingjong[3]; Wallberg, Noah[1 4]; Torres, Sophia[5]; Reyes, Jasmine[2 5]; Sparks, Erin[3 5]; Yandeau-Nelson, Marna D[1 2 5]
[1] Molecular, Cellular & Developmental Biology Graduate Program, Iowa State University, Ames, IA
[2] Genetics, Development and Cell Biology Department, Iowa State University, Ames, IA
[3] Division of Plant Science and Technology, University of Missouri, Columbia, MO
[4] Undergraduate Biology Major, Iowa State University, Ames, IA
[5] Department of Plant and Soil Sciences, University of Delaware, Newark, DE

The hydrophobic cuticle, which covers aerial portions of plants, is the first line of defense against environmental stresses, including drought, UV radiation, temperature, and insects and pathogens. This cuticle is comprised of a cutin polyester matrix that is infused with and laid atop by cuticular waxes, comprised of differing combinations of very long chain fatty acids (VLCFAs), hydrocarbons, aldehydes, alcohols, wax esters, ketones and sometimes terpenoids, depending on the species, organ and stage of development. The biosynthesis of the cuticle can be broken down into five regulatory modules, including fatty acid elongation, cutin monomer synthesis, the decarbonylative and reductive modules within cuticular wax biosynthesis, and transport. We have identified candidate transcription factors (TFs) through multi-omics approaches whose expression levels are associated with cuticle composition. The potential functions of these TFs in the regulation of cuticle biosynthesis and deposition are being evaluated via a synthetic biology approach using a PEG-mediated transient gene expression system within maize and Arabidopsis root protoplasts. Roots are an ideal system to evaluate the regulation of cuticle biosynthesis because they do not synthesize a cuticle for the majority of their lifespan, thus any cuticle production is attributable to the gene expression induced by heterologously expressed candidate TFs. Reverse-transcriptase quantitative PCR (RT-qPCR) was used to evaluate the downstream expression of cuticle genes in each regulatory module after TF induction. Our results show that maize TFs can regulate the homologous cuticle-related genes in Arabidopsis protoplasts. Using this approach, we've shown that both previously reported cuticle TFs and TFs not previously reported to be involved in cuticle formation can activate genes involved in cuticular wax biosynthesis. The TFs validated via this approach are now being expressed in stable Arabidopsis transgenic lines for functional analyses.

Funding acknowledgement: National Science Foundation (NSF)

## P25

## Characterization of transporter genes and their impact on cuticular wax composition on maize silks

(submitted by Noah Walberg <nwalberg@iastate.edu>)
Full Author List: Walberg, Noah[1]; Lane, Madison[2]; Johnson, Joy[3]; Yandeu-Nelson, Marna[1 2 3]
[1] Undergraduate Biology Major, Iowa State University, Ames, IA
[2] Molecular, Cellular & Developmental Biology Graduate Program, Iowa State University, Ames, IA
[3] NSF-REU Program, Iowa State University, Ames, IA

The formation of a physical barrier between an organism and the environment is a critical mode of protection against environmental stress. The cuticle is a hydrophobic barrier that covers the surfaces of aerial organs of land plants and protects against many stresses, including UV light, pests and pathogens, and drought, and can mediate gas exchange and influence plant-insect interactions. Cuticular waxes on the silks of maize are composed primarily of hydrocarbons, minor amounts of alcohols, and very long chain fatty acids. These waxes are synthesized by machinery associated with the endoplasmic reticulum and are then transported to the surface of the cell that interfaces with the environment. These transport mechanisms are not yet fully defined. Using multi-omics approaches, we previously identified genes encoding transporters whose expression levels are associated with cuticle composition on maize silks. To assess whether these genes play a role in cuticular wax transport, we have characterized UniformMu-insertion mutants of genes encoding an Acyl-CoA Binding Protein (ACBP) and a Non-Specific Lipid Transfer Protein (NSLTP). Cuticular wax profiling of silks from wildtype and mutant plants for both the ACBP- and NSLTP-encoding genes showed that total cuticular wax load is lower, with most of the change in wax composition being in hydrocarbon (i.e., alkane and alkene) accumulation. A slight increase in very long chain fatty acids in the NSLTP mutants is also observed, where as the ACBP mutant showed little change in very long chain fatty acid accumulation. These data suggest that these transporters indeed function in transporting cuticular waxes, primarily hydrocarbons, and impact cuticle composition. In the future, these mutants will be evaluated to test whether these changes in cuticular wax load and composition impact the cuticle as a water barrier.

## P26

## Co-expression analysis identifies a transcription factor positively regulates the kauralexin biosynthesis gene *ZmKSL2*

(submitted by Zhijie Shui <zhijie_shui@nwafu.edu.cn>)
Full Author List: SHUI, ZHIJIE[1 2 3]; Bai, Lu[1 2 3]; Zhang, Ruiyu[1 2 3]; Nian, Huiqin[1 2 3]; Wang, Zhe[1 2 3]; Yang, Qin[1 2 3]
[1] College of Agronomy, Northwest A&F University
[2] State Key Laboratory of Crop Stress Biology for Arid Areas
[3] Key Laboratory of Maize Biology and Genetic Breeding in Arid Area of Northwest Region, Ministry of Agriculture, Yangling 712100, China

*ZmKSL2* encodes a diterpene synthase involved in the biosynthesis of kauralexin, one of the major diterpenoid phytoalexins conferring multiple fungal disease resistance in maize. However, it remains unclear how *ZmKSL2* expression is regulated under pathogen attack. Here, we performed a mutual-rank co-expression analysis with 6283 publicly available transcriptome data using *ZmKSL2* as a bait gene. Two transcription factor genes, *ZmWKRY34* and *ZmWRKY36*, were identified as highly co-expressed with *ZmKSL2*. Gene expression analysis showed that *ZmWRKY34* and *ZmWRKY36* were significantly induced by *Cochliobolus heterostrophus* and *Fusarium graminearum*. Transient overexpression of ZmWRKY34 and ZmWRKY36 in *Nicotiana benthamiana* showed that both were mainly localized in the nucleus. Yeast one-hybrid experiments indicated that ZmWRKY34 and ZmWRKY36 were able to bind to the W-box cis-element in the *ZmKSL2* promoter region. Dual luciferase assays confirmed that only ZmWRKY36 significantly activated *ZmKSL2* transcription in maize protoplasts. Further analyses focus on kauralexin biosynthetic genes regulated by *ZmWRKY36* with overexpression lines and EMS mutants. This study provides insights into the mechanism of *ZmKSL2* response to pathogenic stress.

Funding acknowledgement: National Key Research and Development Program of China (#2020YFA0907901)

**P27** @shivreet_kaur

## Comparative analysis of host specificity and pathogen interaction of *Cochliobolus heterostrophus* on maize vs. non-host species

(submitted by Shivreet Kaur <skaur7@ncsu.edu>)
Full Author List: Kaur, Shivreet[1]; Balint-Kurti, Peter[1 2]
[1] Department of Entomology and Plant Pathology, North Carolina State University, Raleigh, NC, USA, 27695
[2] USDA-ARS, Plant Science Research Unit, Raleigh, NC, USA, 27695

Southern leaf blight (SLB), caused by *Cochliobolus heterostrophus*, threatens maize production globally. This necrotrophic fungus typically penetrates the leaf between epidermal cells or through stomates. After penetration the fungus can establish extensive hyphal networks that infiltrate deep into the leaf tissue. While its interaction with maize has been extensively studied, the behavior of *C. heterostrophus* on non-host species such as wheat, sorghum, rice, and tomato remain largely unexplored. The hypothesis driving this study is that *C. heterostrophus* exhibits differential growth and infection strategies when interacting with maize compared to non-host species. We seek to determine whether *C. heterostrophus* recognizes it is on a maize leaf and perceives maize-specific signals and modulates its infection strategy accordingly. To determine whether the pathogen exhibits specific behaviors and growth dynamics on maize compared to non-hosts we are using advanced microscopy techniques like confocal and scanning electron microscopy to visualize and quantify the growth and behavior of pathogen on maize and non-host leaf surfaces. Our preliminary results indicate that the pathogen can germinate on non-host species, albeit at a significantly lower frequency compared to the host species, maize. Furthermore, the penetration efficiency of the fungus is markedly reduced on non-host species relative to maize. Further, by comparing the pathogen's gene expression profiles during infection on host versus non-host species using RNA seq, we hope to identify the molecular pathways that are either activated or suppressed in response to non-host conditions. Understanding the basis of host specificity of *C. heterostrophus* may help us uncover the mechanisms that enable its recognition by non-hosts or conversely the mechanisms that allow the fungus to recognize that it is on a maize leaf and to deploy effective pathogenesis strategies. This knowledge could potentially be useful in developing disease resistant maize varieties.

Funding acknowledgement: United States Department of Agriculture (USDA)

# P28

## Deciphering genetic architecture of stalk lodging resistance using high-density phenotype map in maize

(submitted by Bharath Kunduru <bkundur@clemson.edu>)
Full Author List: Kunduru, Bharath[1]; Bokros, Norbert[2 3]; Tabaracci, Kaitlin[4]; Kumar, Rohit[1 5]; Brar, Manwinder[1]; Machado e Silva, Caique[6 7]; Oduntan, Yusuf[4]; DeKold, Joseph[4]; Woomer, Joseph[3]; Bishop, Rebecca[1]; Verges, Virginia[3]; Stubbs, Christopher[4 8]; Morota, Gota[6]; McMahan, Christopher[9]; Robertson, Daniel[4]; DeBolt, Seth[3]; Sekhon, Rajandeep[1]
[1] Department of Genetics and Biochemistry, Clemson University, SC, USA
[2] Center for Quantitative Genetics and Genomics, Aarhus University, Aarhus, Denmark
[3] Department of Horticulture, University of Kentucky, KY, USA
[4] Department of Mechanical Engineering, University of Idaho, ID, USA
[5] Department of Plant and Environmental Sciences, Clemson University, SC, USA
[6] School of Animal Sciences, Virginia Polytechnic Institute and State University, VA, USA
[7] Department of Agronomy, Federal University of Viçosa, Minas Gerais, Brazil
[8] School of Computer Sciences and Engineering, Fairleigh Dickinson University, NJ, USA
[9] School of Mathematics and Statistical sciences, Clemson University, SC, USA

Stalk lodging undermines crop productivity and causes global annual losses of at least 6 billion USD in maize (Zea mays L.). Poor understanding of plant phenotypes associated with stalk lodging, referred to as intermediate phenotypes, and lack of standardized phenotyping protocols impeded the efforts to enhance genetic resolution of stalk lodging resistance. We generated a multi-environment high-density phenotype map of 15 morphological, geometric, structural, and biomechanical phenotypes on 31,200 stalks of a maize inbred panel, examined the impact of these phenotypes on stalk lodging resistance, and identified underlying genetic loci. Preserving field location of individual plants allowed us to account for spatial effects and refine the phenotype data for statistical analyses. Phenotype analyses revealed significant variation across environments indicating a substantial role of G×E on the phenotypic continuum of intermediate phenotypes. Predictive analytics with machine learning revealed major and minor diameters of internodes and plant height as the key predictors of stalk flexural stiffness. Intermediate phenotypes measured on the lower-most elongated internode showed stronger genetic correlation with stalk flexural stiffness as compared to those measured on the primary ear-bearing internode. Most intermediate phenotypes exhibited low to moderate heritability indicating low genetic tractability and complex genetic inheritance. Genome wide association analyses of the intermediate phenotypes showed candidate genes involved in cell division, plant and ear height, electron transport, membrane structure and transport, oxidoreductase activity, transcription factors, etc. Remarkably, a large proportion of the significant SNPs identified were nested in the non-coding regions of the genome and certain SNPs were shared between different phenotypes indicating pleiotropic regulation of stalk lodging resistance. We are currently evaluating the role of selected candidate genes for their role in stalk lodging resistance in maize.

Funding acknowledgement: National Science Foundation (NSF)

## P29

## Deciphering the role of the cell wall in mycorrhizal symbiosis under low-input conditions in maize

(submitted by Sylvie Coursol <sylvie.coursol@inrae.fr>)
Full Author List: Da Costa, Anaïs[1]; Rigault, Martine[1]; Quilleré, Isabelle[1]; Laurens, Sophie[2]; Bunel, Léo[2]; Le Ru, Aurélie[3]; Le Brusq, Michel[1]; Guillaume, Sophie[1]; Jacquemot, Marie-Pierre[1]; López-Malvar, Ana[1]; Méchin, Valérie[1]; Reymond, Matthieu[1]; Reyt, Guilhem[2]; Dellagi, Alia[1]; Coursol, Sylvie[1]; Horlow, Christine[1]
[1] Université Paris-Saclay, INRAE, AgroParisTech, Institute Jean-Pierre Bourgin for Plant Sciences (IJPB), 78000, Versailles, France
[2] LIPME, Université de Toulouse, INRAE, CNRS, Castanet Tolosan 31326, France
[3] Fédération de Recherche 3450, Plateforme Imagerie, Pôle de Biotechnologie Végétale, Castanet-Tolosan 31320, France

Maize is a cornerstone of the French forage system, with yield and silage feeding value serving as key criteria for the registration of maize hybrid varieties in the official French catalog. Previous studies have shown a direct correlation between silage feeding value and dry matter digestibility, which is primarily influenced by cell wall digestibility. Nitrogen, a critical factor for maize yield, is typically supplied in conventional agriculture through synthetic fertilizers, which are criticized for their environmental impact and rising costs. Therefore, investigating the beneficial effects of arbuscular mycorrhizal fungi on the agronomic performance of maize under conditions of low nitrogen and water availability seems to be a promising approach. In particular, the deposition of cell wall compounds in roots regulates the transport of nutrients, including nitrogen. In this context, our study aims to investigate how altered cell wall composition affects mycorrhizal symbiosis, root development and digestible yield under low-input conditions, using maize mutants with modified lignin content or hemicellulose composition.

Funding acknowledgement: Plant2Pro Carnot institute (CHAMPAGNE project, Agence Nationale pour la Recherche #20 CARN 0024 01), Métaprogramme DIGIT-BIO (PRECURSOR project)

## P30

## Decoding a complex distal non-coding QTL at *TEOSINTE BRANCHED 1*

(submitted by Ankush Sangra <ankush.sangra@uga.edu>)
Full Author List: Sangra, Ankush[1]; Chen, Zongliang[2]; Marand, Alexandre P.[1]; Mendieta, John P.[1]; Minow, Mark A.A.[1]; Clause, Haley N.[1]; Studer, Anthony J.[3]; Gallavotti, Andrea[2]; Schmitz, Robert J.[1]
[1] Department of Genetics, University of Georgia Athens, USA
[2] Waksman Institute of Microbiology, Rutgers University, Piscataway, NJ
[3] Department of Crop Sciences, University of Illinois, Urbana, IL, USA

During domestication from teosinte, maize branching and inflorescence architecture changed drastically, mainly due to increased *TEOSINTE BRANCHED 1 (TB1)* axillary bud expression. Elevated *TB1* expression is due to domestication altering a distal control region (CR) of ~11kb. This domesticated CR has four accessible chromatin regions (ACRs), three of which are consistent with enhancer activity, as they co-occur with *TB1* expression in developing female inflorescence. The final ACR appears consistent with silencer activity, as it is always accessible, even when the *TB1* locus is repressed by H3K27me3 deposition. In contrast, most teosinte contain only three ACRs, as a *Hopscotch* transposon bifurcates one ancestral ACR in domesticated maize. Here, we functionally dissect the *TB1* CR via CRISPR-Cas9 deletion of each CR ACR, *Hopscotch*, and the entire CR. Entire CR deletion phenocopy *TB1* loss of function, confirming that the CR cumulatively enhances *TB1* expression. Deletion of the two ACRs flanking *Hopscotch* caused weak *tb1* branching phenotypes, suggesting additive enhancer action; indeed, these deletions caused reduced axillary bud *TB1* expression, but no expression change in the leaf, where *TB1* is normally repressed. Deletion of the putative silencer ACR caused no overt phenotypic change, yet preliminary results indicate that it changes the chromatin accessibility of the *TB1* promoter. Surprisingly, chromatin at *Hopscotch* is not accessible and *Hopscotch* deletion produced no phenotypic changes – this, along with the presence of this *Hopscotch* insertion in select teosinte accessions, suggest changes other than the *Hopscotch* insertion caused the domestication increase in maize apical dominance. Future exploration will characterize the molecular changes accompanying our ACR deletions in the *TB1* CR and reveal more about the genetics that underpins *TB1* expression control.

Gene / Gene Models described: *tb1*; Zm00001eb054440
Funding acknowledgement: National Science Foundation (NSF), United States Department of Agriculture (USDA)

## P31 @jrrygnzlz

### Developing a high-throughput genotyping assay for rapid identification of multiple edited flowering genes in tropical maize

(submitted by Gerardo Gonzalez <ggg7@hawaii.edu>)
Full Author List: Gonzalez, Gerardo E[1]; Muszynski, Michael[2]; Nocon, Denise[3]
[1] University of Hawai'i at Manoa; Department of Tropical Plant and Soil Sciences, Honolulu, Hawaii, 96822
[2] University of Hawai'i at Manoa; Department of Tropical Plant and Soil Sciences, Honolulu, Hawaii, 96822
[3] University of Hawai'i at Manoa; Department of Tropical Plant and Soil Sciences, Honolulu, Hawaii, 96822

The Genome Engineering To Sustain Crop Improvement (GETSCI) project aims to provide new sources of genetic diversity for breeding by using gene editing to reduce photoperiod sensitivity in tropical maize, which will allow tropical inbreds to flower early in temperate regions. The three key regulatory genes responsible for late flowering of tropical maize under long-day photoperiods are ZmRAP2.7, ZmCCT9, and ZmCCT10. The GETSCI team has generated multiple small insertion and deletion (indel) alleles for these three target genes that are expected to knockout gene function. Our next step is to develop fixed single, double, and triple homozygous knockout genotypes to detect flowering changes under short-day and long-day photoperiods. My project is to develop rapid and high-throughput genotyping assays for our priority single nucleotide indel mutations. This assay is critical for rapidly identifying plants to self-fertilize during the winter nursery and confirming genotypes in preparation for phenotyping trials in summer 2025. I elected to develop and optimize TaqMan genotyping assays that are expected to detect multiple edited alleles in our three target genes. My poster will illustrate my strategy and current progress with developing and testing my genotyping assays on segregating families. By enabling precise and efficient identification of homozygous gene-edited alleles at multiple loci, we will be able to phenotype known fixed genotypes in summer 2025. Our long term goal is to contribute to expanding the adaptability of tropical maize lines to improve breeding globally.

Gene / Gene Models described: *ZmCCT9, ZmCCT10, ZmRAP2.7*; GRMZM2G004483, GRMZM2G381691, GRMZM2G700665
Funding acknowledgement: National Science Foundation (NSF)

## P32

### Developing a system to elucidate genetic factors underlying hypersensitivity responses in maize

(submitted by Jane Mascarenhas <mjane@ksu.edu>)
Full Author List: Mascarenhas, Jane[1]; Liu, Hui[1]; White, Frank[2]; Liu, Sanzhen[1]
[1] Department of Plant Pathology, Kansas State University, Manhattan, KS 66506, USA
[2] Department of Plant Pathology, University of Florida, Gainesville, FL 32611, USA

The hypersensitive response (HR), defined as the rapid death of host cells upon pathogen contact, limiting pathogen colonization and, consequently, provides host resistance. HR typically arises from the interactions between an avirulence (AVR) genes in the pathogen and a resistance (R) gene in the host. However, identifying such AVR/R pairs remains a significant challenge. This project aims to develop an effective approach to facilitate the identification of the R genes interacting with an AVR gene. Specifically, we have established a bacterium-enabled protein delivery system through the maize bacterial pathogen *Xanthomonas vasicola pv. vasculorum* (Xvv). Strain Xvv1601 does not induce hypersensitivity response (HR) responses in most maize varieties. Previous reports showed that some maize varieties contain the R gene *Rxo1* corresponding to the AVR gene *AvrRxo1* from a rice bacterial pathogen. The known interaction was used to test if the delivery of *AvrRxo1* through Xvv1601 can induce HR in maize varieties containing *Rxo1* and, by inference, other similarly functioning AVR/R gene pairs. Preliminary screening revealed HR responses in maize lines B73, A188, HP301, M37W, and Tx303. The screening also identified susceptible responses in Mo17, CML103, Ky21, and Oh7B. Further, the TurboID based proximity labeling for protein tagging was employed to facilitate the identification of proteins interacting with AvrRxo1. We anticipate the approach established in this study will accelerate discovery of novel AVR/R interactions in maize, contributing to improved strategies for crop disease management.

Keywords: *AvrRxo1*, *Rxo1*, hypersensitivity response, TurboID, maize disease resistance, effector proteins.
Gene / Gene Models described: *Rxo1*; Zm00001eb261660
Funding acknowledgement: Plant Genome Research Program(PGRP) NSF

## P33

### Developing an efficient workflow for detecting CRISPR-edited alleles in three target genes that regulate photoperiod response in tropical maize

(submitted by Rina Carrillo <rinamc@hawaii.edu>)
Full Author List: Carrillo, Rina[1]; Hampson, Ella[1]; Uy, Jaclyn[1]; Amore, Teresita[1]; Kang, Minjeong[2]; Lee, Keunsub[2]; Wang, Kan[2]; Nocon, Denise[1]; Muszynski, Michael[1]
[1] University of Hawaii at Manoa; Department of Tropical Plant and Soil Sciences; Honolulu, HI, 96822
[2] Iowa State University; Department of agronomy; Ames, IA, 50011

Advances in gene editing technologies have provided a targeted and rapid approach to genome engineering. These targeted approaches allow for altering genes underlying agronomically relevant traits, including polygenic traits such as photoperiod response in maize. The genetic diversity present in tropical maize germplasm is largely underutilized due to the late-flowering behavior exhibited in temperate environments. To address this bottleneck and promote earlier flowering, the Genome Engineering to Sustain Crop Improvement (GETSCI) project used CRISPR/Cas-9-based gene editing to suppress the photoperiod response in the tropical maize inbred Tzi8. Edits were successfully detected in three target genes that normally function to suppress flowering time, however, developing an efficient and reproducible workflow for high-throughput screening is required. Here, we show our current workflow for initial detection of edits in the genetically transformed tropical maize inbred Tzi8, including subsequent genotyping efforts for selection of fixed edited lines. Transgenic plants containing CRISPR-Cas were obtained by *Agrobacterium* mediated transformation. Tracking of Indels by Decomposition (TIDE) analysis was used to detect the presence of edited individuals from the T0 and T1 generations. Selected individuals containing edits were screened using CAPS markers and Sanger sequencing in the T2 generation. The results from this project open the opportunity for crop improvement worldwide by allowing gene-edited tropical maize germplasm to be used in temperate regions.

Gene / Gene Models described: *ZmCCT9, ZmCCT10, ZmRAP2.7*; GRMZM2G004483, GRMZM2G381691, GRMZM2G700665
Funding acknowledgement: National Science Foundation (NSF)

## P34

### Development of Sorghum BTx642 EMS population

(submitted by Enameguono Olomukoro <eolomuko@purdue.edu>)
Full Author List: Olomukoro, Enameguono E[1 2 3]; Khangura, Rajdeep S[2 3]; Dilkes, Brian[2 3]
[1] Purdue University Interdisciplinary Life Sciences (PULSe), West Lafayette, Indiana 47907 USA
[2] Department of Biochemistry, Purdue University, West Lafayette, Indiana 47907 USA
[3] Center for Plant Biology, Purdue University, West Lafayette, Indiana 47907 USA

Ethylmethanesulfonate (EMS) is an alkylating agent and potent chemical mutagen that induces point mutations (G:C to A:T changes) in the DNA. EMS creates novel genetic diversity within which can result in the manifestation of trait variation including phenotypes not seen or not obvious among natural variants segregating in the species. Mutants derived from EMS populations have contributed to the identification of genes and the molecular understanding of developmental and biochemical phenotypes of interest. Sorghum is a valuable crop for food, forage, and biomass. With high-quality sequenced genomes, diploid genome, and a moderate genome size (810 Mb), sorghum is an excellent system for elucidating gene functions for other complex eukaryotes. We developed a single-seed descent pedigreed sorghum BTx642 EMS mutant library. Mutagenized M1 seeds were propagated individually to M6 seeds through single seed decent. Out of 559 M5 families, visible phenotypes were observed and recorded in 439 families. A wide range of morphological phenotypes were observed in the mutant library. These traits include dwarfs, leaves shape, leaf angle, tillering, rolled leaves, leaf color, leaf number, and dramatic effects on seedlings growth. We will whole genome sequence all phenotyped-mutant lines to provide mutant candidates throughout the genome and use linkage mapping to associate mutations to phenotypes.

Funding acknowledgement: Department of Energy (DOE)

## P35

## Development of efficient haploid inducers of fast flowering mini maize

(submitted by Zhengzhi Zhang <zhangzheng@umsystem.edu>)
Full Author List: Zhang, Zhengzhi[1]; Liu, Hua[1]; Yang, Hua[2]; Albert, Patrice[2]; Char, Si Nian[1]; Birchler, James A[2]; Yang, Bing[1]
[1] Division of Plant Science and Technology, University of Missouri, Columbia, MO 65211
[2] Division of Biological Sciences, University of Missouri, Columbia, MO 65211

Doubled haploid technology enables creating novel, homozygous lines in one generation, speeding up creation of true-breeding germplasm in crop breeding. Haploid inducers (HI) can be created through modifying the *PLA1/NLD/MTL* and *DMP* genes, and produce maternal haploids when the HI, used as the pollen parent, is crossed to other maize genotypes. In the present study, Fast Flowering Mini Maize (FFMM) was used for CRISPR-Cas9 based gene-editing of the *ZmMTL* and *ZmDMP* genes. To aid identification of haploid individuals, *GFP* and *RUBY* markers were incorporated into the edited lines, creating HI lines with various combinations of haploid induction genes. Progeny derived from Mo17 pollinated with these HI lines were initially screened for haploids by the absence of GFP fluorescence in seedling roots. HI lines carrying only the CRISPR-Cas9 edited ***mtl*** allele ($mtl_e$) or the **stock6 *mtl*** allele ($mtl_{s6}$) produced haploids at rates of 3.3% and 6.3%, respectively. In contrast, HI lines harboring both $mtl_e$ and the edited ***ZmDMP*** allele ($mtl_e$/*dmp*) or $mtl_{s6}$ and ***ZmDMP*** edited allele ($mtl_{s6}$/*dmp*) exhibited significantly higher haploid induction capabilities, with haploid induction rates of 15.0% and 14.0%, respectively. Haploid plants subjected to nitrous oxide gas treatment (2 days at 600 kPa) at the six-leaf stage developed fertile sectors on the tassels. Additionally, haploids that did not undergo chromosomal doubling treatment were still able to produce normal kernels when pollinated by normal pollen, likely due to meiotic failure or spontaneous genome doubling during the developmental stages leading to the ear. Due to economy of space and 30 days or less to flowering under standard greenhouse conditions, HI in Fast Flowering Mini Maize will facilitate haploid induction in maize.Supported by NSF PGRP Award, IOS-2221891.

Funding acknowledgement: National Science Foundation (NSF)

## P36

## Endogenous gene activation of a MYB transcription factor by CRISPRa drives wax biosynthesis regulation in maize

(submitted by Hui Liu <huil@ksu.edu>)
Full Author List: Liu, Hui[1]; Tamang, Tej Man[1]; Mascarenhas, Jane[1]; Liu, Sanzhen[1]
[1] Department of Plant Pathology, Kansas State University, Manhattan, KS 66506-5502, USA

Artificial modulation of gene expression can rewire biological processes for desired traits. CRISPR-based activation (CRISPRa), utilizing dead Cas9 (dCas9) fused with synthetic transcriptional activators, offers a programmable system for transcriptional activation of endogenous genes. Here, we adapted a CRISPRa approach, dCas9-TV, and demonstrated its effectiveness by testing dozens of genes in maize protoplasts. In particular, dCas9-TV was employed to activate *gl3*, a gene encoding a MYB transcription factor regulating cuticular wax biosynthesis. RNA-seq confirmed the significant activation of *gl3* by dCas9-TV and revealed the subsequent GL3-mediated upregulation of most known genes in cuticular wax pathways, underscoring the functional impact of *gl3* activation. Furthermore, integration with Cut&Tag, a technique for profiling protein-DNA interactions, identified direct GL3 target genes, supported by binding domain analyses and transactivation assays. This study establishes dCas9-TV as a robust platform for activating single or multiple genes at endogenous loci, overcoming the limitations of conventional methods and enabling detailed exploration of transcriptional complexity and gene regulation in maize. **Keywords**: maize, transcriptional activation, CRISPRa

Gene / Gene Models described: *gl3*; Zm00001eb195850
Funding acknowledgement: National Science Foundation (NSF), United States Department of Agriculture (USDA), Department of Energy (DOE)

## P37

## Engineering maize mosaic virus for efficient virus induced gene silencing and virus induced DNA-free genome editing in maize: a potential ally for maize improvement

(submitted by Cesar Xavier <cdinizx@ncsu.edu>)
Full Author List: Xavier, César A. D.[1]; Singh, Deepjyoti[1]; Whitfield, Anna E.[1]
[1] North Carolina State University, Raleigh, North Carolina, USA, 27606

Viral vectors are a versatile and powerful tool for functional genomics and have great potential to be used for crop improvement through either transient or stable genetic modifications. Although several viral vectors based on positive-sense RNA viruses have been previously developed for maize, they have limited use as they are usually unstable and have limited cargo capacity. Negative-sense RNA viruses including plant rhabdoviruses are the most desirable systems as they exhibit greater genetic stability and can carry large cargoes. We have recently developed a viral vector for the negative-sense RNA plant rhabdovirus, maize mosaic virus (MMV), which performs superiorly compared to viral vectors currently available for maize. Here, we have further engineered MMV-based vectors to perform virus-induced gene silencing and virus induced DNA-free genome editing in maize. By engineering a MMV vector expressing a hairpin construct having an antisense intron 2 ST- LS1 as a loop and a 400 nt self-complementary stem targeting *Zea mays Phytoene desaturase* (*ZmPDS*) transcript, we were able to obtain a completely photobleached maize plant. Analysis of *ZmPDS* transcript abundance demonstrated significant silencing 30 days after virus inoculation. We have also engineered MMV to express Cas nuclease and guide RNAs targeting exonic regions of *ZmPDS* to perform DNA-free genome editing mediated by a viral vector. Genotyping analysis based on amplicon sequencing demonstrated that targeted sites were edited with indel frequencies ranging from 0% to 2.4%. This is the first negative-strand viral vector for efficient use in maize and it is a powerful tool to perform gene expression, gene silencing and DNA-free genome editing in one of the most important crops. The MMV system has great potential to be used for maize improvement.

Funding acknowledgement: United States Department of Agriculture (USDA)

## P38

## Expression of the solanaceous immune receptor FLS3 in maize protoplasts

(submitted by Justin Larkin <jtlarkin@illinois.edu>)
Full Author List: Larkin, Justin T.[1]; Zimmerman, David J.[1 2]; Refi Hind, Sarah[1]
[1] Department of Crop Sciences, University of Illinois Urbana-Champaign, Urbana, IL, USA, 61801
[2] Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, USA, 11724

FLAGELLIN-SENSING 3 (FLS3) is a cell-surface immune receptor found in some members of the Solanaceae family and is responsible for perception of infection by a variety of plant pathogens such as *Xanthomonas euvesicatoria pv. euvesicatoria* (Xe) and *Xanthomonas euvesicatoria pv. perforans* (Xp) (Hind et al., 2016; Malvino et al., 2021). These pathogens are the causal agents of bacterial spot in pepper and tomato. FLS3 binds to the flagellin epitope flgII-28 to initiate downstream immune responses. While the disease resistance conferred by FLS3 to *Xanthomonas* pathogens in tomato and pepper is well understood (Malvino et al., 2021), it is not known whether FLS3 could function in a different species such as *Zea mays*. Bacterial leaf streak of maize is caused by the pathogen *Xanthomonas vasicola pv. vasculorum* (Xvv) (Liu et al., 2023). The flgII-28 epitope from Xvv shares a 92.9% and a 96.4% similarity to those present in Xe and Xp, respectively. Preliminary in silico protein structure prediction models show that the flgII-28 from Xvv can bind to the same predicted ligand binding domain as those from Xp and Xe. Due to these similarities, we hypothesize that transfecting maize protoplasts with the FLS3 receptor would allow for elicitation of an immune response when exposed to flgII-28 peptides. Hind, S. R. et al. (2016). *Nature Plants*: https://doi.org/10.1038/nplants.2016.128 Malvino, M. L. et al. (2021). *Molecular Plant-Microbe Interactions:* https://doi.org/10.1094/MPMI-08-21-0211-R Liu, Z. et al. (2023). *Phytopathology:* https://doi.org/10.1094/PHYTO-11-23-0423-SA

Funding acknowledgement: National Science Foundation (NSF)

## P39

### *Expression variation in Glossy15 is associated with harvest index in maize*

(submitted by Maruti Nandan Rai <mnrai@illinois.edu>)
Full Author List: Rai, Maruti Nandan[1]; Zhang, Wei[1]; Moose, Stephen[1]
[1] University of Illinois Urbana-Champaign, Urbana, IL, USA, 61801

Maize grain yields in the US Corn Belt have risen 6-fold during the past century, due in part to increasing harvest index, the ratio of harvested grain to total biomass. Although larger ears with more and heavier kernels certainly contribute to higher harvest index, the potential influence of changes in vegetative biomass has not been directly explored. We demonstrate here that variation in the expression of the APETALA2-class transcription factor *Glossy15* (*Gl15*), a key regulator of vegetative phase change, also modulates harvest index. Historical maize breeding has shortened the duration of the juvenile vegetative phase, which is also associated with fewer vegetative nodes. Two classes of functional *Gl15* haplotypes exist in maize germplasm, those with a second *microRNA172* (*miR172*) target site that are more weakly expressed compared to haplotypes that lack the secondary *miR172* site. The weaker *Gl15* haplotypes are associated with a shorter juvenile phase and higher harvest index in populations of maize hybrids, although the two classes of haplotypes appear to be under balancing selection. Cis-genic introduction of a native *Gl15* haplotype lacking the secondary *miR172* target site increases *Gl15* expression, delays vegetative phase change, and reduces harvest index in multiple hybrid backgrounds. Conversely, near-isogenic hybrids homozygous for *gl15* loss-of-function mutations exhibit higher harvest index via reductions in stalk biomass while maintaining grain yield. These findings indicate that selection for a shorter juvenile phase, mediated by native *Gl15* alleles with greater sensitivity to *miR172*-mediated repression, offers a novel opportunity to further improve maize harvest index.

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

## P40

### Flood-induced insect resistance in maize involves flavonoid-dependent salicylic acid induction

(submitted by Zachary Gorman <zachary.gorman@usda.gov>)
Full Author List: Gorman, Zachary[1]; Liu, Hui[1]; Sorg, Ariel[1]; Grissett, Katherine[1]; Yactayo-Chang, Jessica[1]; Li, Qin-Bao[1]; Rivers, Adam[3]; Basset, Giles[2]; Rering, Caitlin[1]; Beck, John[1]; Hunter, Charles[1]; Block, Anna[1]
[1] Chemistry Research Unit, United States Department of Agriculture-Agricultural Research Service, Gainesville, FL, USA
[2] Horticultural Sciences Department, University of Florida, Gainesville, FL, USA
[3] Genomics and Bioinformatics Research Unit, United States Department of Agriculture-Agricultural Research Service, Gainesville, FL, USA

Plants have evolved the ability to respond to a diverse range of biotic and abiotic stresses. Often, combining these stresses multiplies the challenge for the plants, but occasionally the combined stress can induce unexpected synergistic defenses. In maize, combined flooding and herbivory induces a salicylic acid (SA)-dependent defense against *Spodoptera frugiperda* (fall armyworm). In this study we used RNAseq and metabolic profiling to show that flavonoids are involved in maize response to combined flooding and herbivory. To assess the role of flavonoids in flood-induced *S. frugiperda* resistance, we analyzed the maize *idf* mutant that has compromised expression of chalcone synthase, the first enzyme in flavonoid biosynthesis. This flavonoid-deficient mutant was compromised both in flood-induced *S. frugiperda* resistance and in SA accumulation. These data revealed an unexpected requirement for flavonoids in SA induction. In contrast to *idf*, the flavonoid 3' hydroxylase mutant, *pr1*, showed enhanced SA accumulation after combinatorial treatment, which closely correlated with elevated levels of select flavonoids and the dihydroflavonol reductase, anthocyaninless1 (*a1*) mutant, was unaffected in its SA-induction. These data indicate that flavonoids likely derived from dihydrokaempferol, apigenin, or luteolin play a role in flood-induced SA accumulation and *S. frugiperda* resistance.

Gene / Gene Models described: *c2*, *pr1*, *a1*, *fnsi-1*, *fnsi-2*; Zm00001d052673, Zm00001d017077, Zm00001d044122, Zm00001d029744, Zm00001d027423
Funding acknowledgement: United States Department of Agriculture (USDA)

## P41

# Gene expression and circadian rhythm differences between temperate and tropical maize inbreds in response to photoperiod

(submitted by Joseph DeTemple <josephdetemple@gmail.com>)
Full Author List: DeTemple, Joseph[1]; Hinrichsen, Jacob[1]; Li, Dongdong[1]; Schoenbaum, Greg[1]; O'Rourke, Jamie[1 2]; Graham, Michelle[1 2]; Li, Xianran[3]; Muszynski, Michael[4]; Yu, Jianming[1]
[1] Department of Agronomy, Iowa State University, Ames, IA, US
[2] USDA-ARS Corn Insects and Crop Genetics Research Unit, Ames, IA, US
[3] USDA-ARS, Wheat Health, Genetics, and Quality Research, Pullman, WA, US
[4] Department of Tropical Plant and Soil Sciences, University of Hawai'i at Mānoa, Honolulu, HI, US

The floral transition of maize, where the shoot apical meristem switches from vegetative to reproductive growth, is controlled by networks of genes that capture, process, and interpret environmental signals such as photoperiod. The critical gene networks underlying the response to photoperiod have been generally mapped out through quantitative and molecular studies. However, a more complete picture of these networks is critical for fully understanding the responses of temperate, tropical, and mixed maize lines to inductive and non-inductive photoperiods. We set out to answer three questions: 1) which genes are the most important within the photoperiod pathway of the maize flowering time gene network, 2) which developmental stages are the most important for the expression of these photoperiod genes, and 3) what times of day are the most important for capturing the expression of these key genes. To answer these questions, we grew the inbred lines B73, Oh43, Mo17, TX303, Ki3, CML277, CML52, and Tzi8 under 15h/9h light/dark conditions in growth chambers, collected leaf tissue at V2-V10 stages at 3 different timepoints (6am, 2pm, and 10pm), and ran qPCR reactions for a subset of 10 genes of interest (prrtf1, cca1, toc1, gi1, col3, elf3.1, elm1, mads69, pebp8, and cct1) that lie within the circadian rhythm and photoperiod pathways of the maize flowering time gene regulatory network. Phenotypes collected throughout the experiment include V-stage date, flowering time, plant height, leaf number, leaf area, and biomass measurements of different plant organs. General circadian rhythm patterns are presented, along with genotypic differences. Overall, this experiment deepens our understanding of which genes are the most important in regulating flowering time differences between temperate and tropical maize lines, what developmental stages are the most critical for the floral transition, and what times of day the key genes are most actively expressed. This experiment will be continued in the future by growing the same genotypes under contrasting short-day 12h/12h light/dark photoperiod conditions.

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## P42

## Generating crossovers at targeted sites in the maize genome

(submitted by Olga Zimina <oz32@cornell.edu>)
Full Author List: Zimina, Olga[1]; Wang, Minghui[1]; Pawlowski, Wojtek[1]
[1] Cornell University, Ithaca, NY, 14850, USA

We are developing tools to direct meiotic recombination to specific genomic sites in maize. These tools will enhance breeders' ability to generate plants with desired features by involving genes that rarely recombine to stack multiple traits or break undesirable genetic linkages. To do it, we are targeting meiotic double-strand breaks (DSBs), which initiate meiotic recombination, to three distinct genomic sites on chromosome 3 in transgenic maize lines expressing a recombinant dCas9:SPO11-1 protein under the control of the constitutive *Ubi1* promoter along with multiplexed gRNAs. The three sites are located in telomeric, interstitial, and pericentromeric regions, which exhibit varying recombination rates in wild-type plants. Crossover (CO) frequency was measured by pollen typing using digital droplet PCR (ddPCR). Pollen grains were collected from transgenic hybrid plants selected to show robust expression of the full-length recombinant protein and the gRNA cassettes. Pollen nuclei were isolated by flow cytometry and genotyped using allele-specific TaqMan probes. We found increased irecombination rates at the targeted telomeric site in the transgenic plants compared to wild-type control. However, no significant differences in CO frequency were observed at the interstitial and pericentromeric regions. This outcome suggests the involvement of additional factors in the formation of COs, beyond the presence of DSBs, which we aim to explore in future studies. This study provides insights into the mechanisms underlying DSB and CO formation and opens the way for developing methods to target meiotic recombination to specific genome location to improve crop breeding.

Gene / Gene Models described: *ZmSPO11-1*; GRMZM2G129913, Zm00001eb214790
Funding acknowledgement: Meiogenix company

## P43

## Genetic architecture of specialized metabolites in sorghum and maize

(submitted by Elliot Braun <braunell@msu.edu>)
Full Author List: Braun, Elliot[1 2]; Thompson, Addie[2 3]; Grotewold, Erich[4]; VanBuren, Robert[2 5]; Schnable, James[6]
[1] Genetics and Genome Sciences Program, Michigan State University, East Lansing, MI 48823, USA
[2] Plant Resilience Institute, Michigan State University, Michigan State University, East Lansing, MI USA
[3] Department of Plant, Soil and Microbial Sciences, Michigan State University, East Lansing, MI USA
[4] Department of Biochemistry and Molecular Biology, Michigan State University, East Lansing, MI 48823, USA
[5] Department of Plant Biology, Michigan State University, East Lansing, MI 48823, USA
[6] Center for Plant Science Innovation & Department of Agronomy and Horticulture, University of Nebraska-Lincoln, Lincoln, NE USA

In the face of a rapidly changing climate and selection for yield, alleles are often lost in elite crop varieties, leading to a reduction in the number of compounds available in the plant toolbox to mitigate the effects of stress. The identification of specialized metabolites from diverse genetic populations can open the door for discovering their role in plant defense and resilience. The accumulation of these compounds can then be compared with agronomic traits to inform an optimal trade-off between growth and defense. In this study we conducted untargeted metabolomics on sorghum grain from the Sorghum Association Panel to identify over 20,000 metabolic signatures. We then filtered the highest confidence signals and used each of these compounds as phenotypes in a genome-wide association study (GWAS) to identify regions of the sorghum genome related to their accumulation. These loci were compared to similar metabolomic datasets from maize kernel and seedling tissue, and we conducted cross-species comparative GWAS to refine our candidate gene lists. Shared regions of these genomes associated with the same metabolites can increase confidence in candidate genes while unique regions and metabolites can highlight metabolic signatures of domestication or selection pressures. The findings can inform breeding programs for desired metabolic features which have the potential to increase plant resilience and food security.

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

## P44

## Harnessing male sterility to accelerate genetic research in the C4 model *Setaria viridis*

(submitted by Hui Jiang <hjiang@danforthcenter.org>)
Full Author List: Jiang, Hui[1]; Myers, Erik[2]; Millman, Britney[1]; Voytas, Daniel F.[2]; Baxter, Ivan[1]
[1] Donald Danforth Plant Science Center; 975 N Warson Rd; St. Louis, MO, 63132
[2] Department of Genetics, Cell Biology and Development, University of Minnesota, 321 Church St SE, Minneapolis, MN 55455

*Setaria viridis* is an emerging genetic model for C4 crops such as maize and sorghum, particularly among panicoid grasses, due to its small size, short life cycle, high seed production, and sequenced genome. Its high transformability via *Agrobacterium tumefaciens* enables stable transgene insertion and CRISPR/Cas9 mutagenesis, facilitating gene function studies and trait stacking. However, its predominantly self-pollinating nature makes genetic crosses challenging, limiting its full research potential. The current crossing protocol relies on heat emasculation, which is labor-intensive and requires significant expertise. To overcome this barrier, we developed a male-sterile line by targeting the *Setaria viridis* ortholog of *Setaria italica No Pollen 1 (SiNP1)*, a gene encoding a glucose methanol oxidoreductase (GMCO) essential for pollen exine formation. In *S. italica*, *SiNP1* knockouts result in a no-pollen phenotype. We targeted *SiNP1* in *S. viridis* ME034 using Cas9 mediated editing. Among 44 $T_0$ transformation events, 40 carried deletions at the target site. We backcrossed male-sterile $T_0$ plants with ME034V and established a stable male-sterile line with a homozygous 57-bp deletion, easily genotyped via PCR and agarose gel electrophoresis. Using this male-sterile line, we developed a simple and highly efficient crossing protocol for *S. viridis* that eliminates the need for emasculation and facilitates manual pollination. This advancement will accelerate both forward and reverse genetic research, enabling more efficient genetic mapping, trait introgression, and functional genomics, further establishing *S. viridis* as a premier C4 model system. Additionally, we investigated pollen flow and quantified outcrossing rates under controlled greenhouse conditions, providing valuable insights into optimal bagging timing to prevent outcross contamination during seed production.

Funding acknowledgement: Department of Energy (DOE)

## P45

## How maize eliminates an apparent toxic by-product - Identification and functional characterization of the $C_4$-ketone products of homoterpene biosynthesis in *Zea mays*

(submitted by Joerg Degenhardt <joerg.degenhardt@pharmazie.uni-halle.de>)
Full Author List: Jochimsen, Christina M[1]; Schaff, Claudia[1]; Degenhardt, Joerg[1]
[1] Martin Luther University Halle-Wittenberg; Halle, D-06120 Germany

Maize commonly emits homoterpenes such as the $C_{11}$-compound (3*E*)-4,8-dimethyl-1,3,7-nonatriene (DMNT). Despite its common occurrence, a functional role of DMNT has been identified only in tritrophic interactions between maize, lepidopteran larvae and parasitic wasps. Our previous work has identified the biosynthetic pathway of DMNT in *Zea mays*, which proceeds in two enzymatic steps. First, the terpene synthase TPS2 catalyzes the formation of the tertiary $C_{15}$-alcohol (*E*)-nerolidol from the ubiquitous farnesyl diphosphate substrate. The subsequent oxidative degradation of (*E*)-nerolidol is catalyzed by a cytochrome P450 monooxygenase, CYP92C5. While this oxidative elimination step has not been characterized in detail, the formation of a $C_4$ fragment has been postulated. We identified this by-product of homoterpene biosynthesis and aim to determine its fate *in planta*.CYP92C5 was heterologously expressed in *Saccharomyces cerevisiae* and the enzyme was incubated with the substrate (*E*)-nerolidol *in vitro*. The butane derivatives methyl vinyl ketone (MVK) and methyl ethyl ketone (MEK) were detected. While MEK was stable under *in vitro* conditions, MVK was converted to MEK. This conversion was also observed in plant systems and is probably catalyzed by reductase activities. It is necessary for plants to eliminate MVK due to the toxicity of the α,β-unsaturated carbonyl structure. We identified putative reductases in *Zea mays* with an expression pattern similar to that of *cyp92c5*. To determine whether these reductases catalyze the detoxification step, activity assays were performed.

Funding acknowledgement: German Research Foundation (DFG)

## P46

# How to control meiotic recombination: The role of chromatin state and DSB resection in determining crossing-over locations in maize

(submitted by Mateusz Zelkowski <mz548@cornell.edu>)
Full Author List: Zelkowski, Mateusz[1]; Bukowski, Robert[1]; Wang, Minghui[1]; Pawlowski, Wojtek[1]
[1] Cornell University, Ithaca, NY, USA

Meiotic recombination plays critical roles in shaping genome composition, driving adaptation, and advancing plant breeding. Understanding where crossovers (COs) occur on the genome can significantly improve breeding efficiency. Knowledge of how to control meiotic recombination opens new opportunities for creating plants with desired trait combinations. Meiotic recombination is initiated by the formation of double-strand breaks (DSBs) in chromosomal DNA. In most species, meiotic DSBs occur far more frequently than COs. The regulatory mechanisms behind the transition from DSBs to COs are not well understood. To explore this question, we performed nucleotide-resolution mapping of recombination intermediates in maize. Our analysis revealed that DSBs with resection spans between 500 and 1500 bp were more likely to result in COs. We tracked CO intermediates at different meiosis stages using chromatin immunoprecipitation (ChIP) with a CO marker protein MLH3. In early prophase I, CO intermediates were distributed along entire chromosomes, reflecting DSB distribution. However, at pachytene and diplotene, CO intermediates showed patterns that closely resembled the final CO distribution, where most COs are located close to chromosome ends. Additionally, chromatin analysis indicated that CO sites in early prophase exhibited DNA methylation patterns similar to the genome average, while late prophase recombination sites were hypomethylated. This phenomenon implicates chromatin as the major factor in determining CO locations. We further investigated the impact of chromatin on CO formation by analyzing *ddm1* and *zmet2* mutants, both of which show reduced DNA methylation and increased CO numbers. We found that the CO landscape differed between the mutants: in *ddm1*, COs were concentrated at chromosome ends, while in *zmet2*, they were more frequently in pericentromeric regions. These CO distribution differences were linked to the nucleosome density patterns in the two mutants. Altogether, our studies show that major determinant of COs are DSB resection, DNA methylation and nucleosome density. Manipulating chromatin state allows increasing the CO number and redirecting them to new chromosome sites.

## P47

### Impact of phosphate-solubilizing bacteria inoculation on maize yield, root system architecture and microbiome

(submitted by Sylvia Morais de Sousa Tinoco <sylvia.sousa@embrapa.br>)
Full Author List: Santos, Jonnathan W M[1]; Oliveira, Raquel G[2]; Avelar, Joao V S[3]; Santos, Flavia C[2]; Oliveira-Paiva, Christiane A[2]; Gomes, Eliane A[2]; Lana, Ubiraci G P[2]; Tinoco, Sylvia M S[1 2 3]
[1] Genomics for Climate Change Research Center (GCCRC) Campinas, SP, Brazil, 13083-875
[2] Embrapa Maize and Sorghum, Sete Lagoas, MG, Brazil, 35701-970
[3] Federal University of São João del-Rei (UFSJ), São João del-Rei, MG, Brazil, 36301-158

The global use of bioinoculants in sustainable agriculture is increasing. BiomaPhos®, a bioinoculant composed of two phosphorus-solubilizing *Bacillus* strains (CNPMS B2084 and CNPMS B119), has been shown to enhance maize yield in Brazil. This study aimed to evaluate the effects of BiomaPhos® inoculation and phosphate fertilization on maize yield, root system architecture and microbiome. Field experiment was conducted in low-phosphorus (P) soil at the Embrapa Maize and Sorghum Experimental Station in Brazil during the 2022/2023 season. A maize hybrid was cultivated under different sources and doses of phosphate fertilizers: no addition of phosphorus fertilizer (P0), rock phosphate (RockP), and triple superphosphate (TSP) at 120 kg $P_2O_5$ $ha^{-1}$, with and without BiomaPhos® inoculation. Root system architecture (RSA) was analyzed using the Digital Imaging of Root Traits (DIRT 5.2) platform. Genetic diversity was assessed at flowering time using Terminal Restriction Fragment Length Polymorphism (T-RFLP), and taxonomic groups were identified with MiCA3 software. Significant differences in yield were observed among treatments, with the highest yields recorded for the combination of TSP fertilizer at 120 kg $ha^{-1}$ and BiomaPhos® inoculation. Correlation analysis revealed a significant positive relationship between maize root area, width, and angle, with root angle showing a strong positive correlation with yield. Root area and width were notably greater in treatments with 120 kg $ha^{-1}$ of TSP. Acid phosphatase activity was highest with RockP fertilizer at 120 kg $ha^{-1}$, while alkaline phosphatase activity peaked in non-inoculated treatments with RockP. The inoculation significantly influenced the soil bacterial community, while fertilization primarily impacted the RSA. The bacterial families Streptomycetaceae, Bacillaceae, and Microbacteriaceae were the most abundant across treatments, with Streptomycetaceae being particularly dominant in RockP-inoculated treatments. Diversity indices (Simpson and Shannon) were negatively correlated with acid and alkaline phosphatase activities. Overall, the findings demonstrate that BiomaPhos® inoculation altered the soil microbial community, while phosphate fertilization modulated root architecture. The combined use of P-solubilizing *Bacillus* strains inoculation and phosphate fertilization enhances maize yield, offering a sustainable strategy for improving agricultural performance.

Funding acknowledgement: Embrapa, Finep, Fapemig, CNPq, Capes

## P48

### Implementation of a lab-scale highly efficient CRISPR cas-9 gene editing and genotyping system in maize.

(submitted by Zackariah Ellington <zfleming3@unl.edu>)
Full Author List: Ellington, Zackariah[1 2 3]; Wang, Zhen[1 2]; Yang, Jinliang[1 2]
[1] Department of Agronomy and Horticulture, University of Nebraska - Lincoln; Lincoln; Nebraska; 68583
[2] Center for Plant Science Innovation, University of Nebraska - Lincoln; Lincoln; Nebraska; 68583
[3] Complex Biosystems Program, University of Nebraska - Lincoln; Lincoln; Nebraska; 68583

Understanding the genotype to phenotype relationship is a major research topic in plant science and plant genetics. A key tool in reverse genetic studies is the targeted mutagenesis of genes of interest. In this study, we implemented a high efficiency gene transformation system for generating CRISPR Cas-9 edits in maize utilizing the morphogenic factors Baby Boom and GRF-GIF (GGB). The GGB system showed greatly increased transformation efficiency using the inbred line B104 (16% regeneration rate) when compared to published literature. We did not observe the major morphogenic changes typically associated with the use of morphogenic factors. In the meantime, we developed a multiplexed genotyping method to identify the gene edits through next-generation sequencing. This highly efficient and cost-effective transformation and genotyping system makes small-scale, lab-based gene editing more attainable, enabling the advancement of plant scientific research.

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

**P49** @minorallele.bsky.social

## Introgression of a Mexican highland chromosomal inversion into temperate maize accelerates flowering, promotes growth, and modulates a cell proliferation gene network.

(submitted by Fausto Rodríguez-Zapata <frodrig4@ncsu.edu>)
Full Author List: Rodríguez-Zapata, Fausto[1 2]; Tandukar, Nirwan[1 2]; Barnes, Allison[1]; Stokes, Ruthie[1]; Aragón-Raygoza, Alejandro[1]; Li, Meng[3]; Pérez-Limón, Sergio[3]; Perryman, Melanie[3]; Piñeros, Miguel A.[4]; Strable, Josh[1 2]; Runcie, Daniel[5]; Sawers, Ruairidh J.[3]; Rellán-Álvarez, Rubén[1 2]
[1] Molecular and Structural Biochemistry, North Carolina State University, Raleigh, North Carolina, USA
[2] Genetics and Genomics, North Carolina State University, Raleigh, North Carolina, USA
[3] Department of Plant Science, The Pennsylvania State University, University Park, PA, USA
[4] Robert W. Holley Center for Agriculture and Health, USDA-ARS, Ithaca, NY, USA
[5] Department of Plant Sciences, University of California, Davis, Davis, CA, USA

Inv4m is a chromosomal inversion prevalent in traditional maize varieties adapted to the cold and often phosphorus-deficient Mexican highlands. Field trials throughout Mexico have shown that, when grown at high elevations, plants carrying the inversion flower faster and have greater yield than plants without it. Although growth chamber experiments indicate that Inv4m regulates the expression of photosynthesis-related genes in response to cold, we have yet to know the genes responsible for the adaptive effects of Inv4m in the field. To identify Inv4m-regulated genes that underlie enhanced development in the field, we bred B73-based Near Isogenic Lines (NILs) with either the inversion or the standard karyotype. We then grew these NILs in phosphorus-sufficient and deficient soils to test whether Inv4m contributes to local adaptation through enhanced phosphorus stress response. We measured plant reproductive and vegetative traits, phosphorus, lipids, and gene expression in the leaves. Plants showed classical responses to phosphorus starvation, including decreased phosphorus and biomass accumulation, delayed flowering, and a switch from phospholipid to glycolipid production. Notably, Inv4m plants flowered earlier and grew taller regardless of phosphorus availability. While increased leaf age and phosphorus deficiency resulted in genome-wide expression changes, Inv4m's effects were predominantly confined to genes within the inversion. Our analyses suggest that Inv4m introgression modulates a trans-coexpression network enriched in cell proliferation and flower development genes, which includes DNA replication fork protein coding genes (pcna2, mcm5), histone demethylases (jmj2, jmj21), and the FT florigen homolog zcn26. By cross-referencing with a list of candidates from the literature, we found other Inv4m-regulated genes associated with flowering time and plant height. In a complementary greenhouse experiment we noticed that Inv4m plants had longer shoot apical meristems, supporting its effect on organ development. These findings provide insights into Inv4m's role in highland adaptation through the coordinated expression of a developmental gene network.

Funding acknowledgement: National Science Foundation (NSF), United States Department of Agriculture (USDA), Science and Technologies for Phosphorus Sustainability (STEPS)

## P50

## LYSDH overexpression increases drought tolerance in maize

(submitted by Ana Lúcia Pinheiro <anampinh@gmail.com>)
Full Author List: Mendes Pinheiro, Ana Lúcia[1 2]; Pinto, Maísa de Siqueira[1]; Bruno, Maria Helena Faustinoni[1 2]; da Silva, Viviane Cristina Heinzen[1]; Pereira, Helcio Duarte[1]; Gerhardt, Isabel Rodrigues[1 3]; Dante, Ricardo Augusto[1 3]; Yassitepe, Juliana Erika de Carvalho Teixeira[1 3]; Arruda, Paulo[1]
[1] Genomics for Climate Change Research Center (GCCRC), State University of Campinas (UNICAMP), Brazil.
[2] Biology Institute, Universidade Estadual de Campinas (UNICAMP), Campinas, Brazil
[3] Embrapa Agricultura Digital, Campinas, Brazil

Lysine catabolism plays a role in the stress response in both eukaryotic and prokaryotic organisms. There are two catabolic pathways for lysine that lead to the production of aminoadipate semialdehyde (AASA): the saccharopine pathway (SacPath) and the lysine dehydrogenase (LYSDH) pathway. In the SacPath, lysine is converted into saccharopine by lysine-ketoglutarate reductase (LKR), which is then transformed into glutamate and AASA by saccharopine dehydrogenase (SDH). In contrast, the LYSDH pathway, which is exclusive to prokaryotes—especially extremophiles—directly converts lysine into AASA. In both pathways, AASA is subsequently converted into α-aminoadipate by AASA dehydrogenase (AASADH). Both SacPath and LYSDH are induced by abiotic stress and are associated with osmolyte accumulation and aldehyde detoxification. We generated transgenic maize plants that overexpress LYSDH derived from the extremophilic bacteria Ruegeria pomeroyi and Geobacillus stearothermophilus. The results indicated that plants overexpressing LYSDH exhibited higher survival rates and increased shoot and root biomass compared to wild-type plants, without compromising overall growth under well-watered conditions. These findings support the idea of manipulating lysine catabolism to develop crops that are more resilient to the challenges of climate change.

Funding acknowledgement: FAPESP (Brazil)

## P51

## Methylation-responsive promoters for engineering gene expression

(submitted by Jonathan Gent <gent@uga.edu>)
Full Author List: Gent, Jonathan I[1]; Sun, Yirui[2]; Dawe, R Kelly[1 3]; Higgins, Kaitlin M[4]; Anderson, Sarah N[4]
[1] Department of Plant Biology, University of Georgia; Athens, GA, USA 30602
[2] Institute of Bioinformatics, University of Georgia; Athens, GA, USA 30602
[3] Department of Genetics, University of Georgia; Athens, GA, USA 30602
[4] Department of Genetics, Development, and Cell Biology, Iowa State University; Ames, Iowa, USA 50011

DNA methylation is a central feature of heterochromatin, where it functions together with histone modifications and other factors to repress transcription. It does so primarily in the context of genome defense, and so its repression is characteristically stable and keeps potentially harmful DNA repressed throughout development. In contrast, developmentally responsive gene regulation depends on chromatin modifications and transcription factors that are characteristically dynamic. We are investigating an unusual mechanism for developmental gene regulation that targets a set of pollen-specific maize genes, which we hypothesize to layer DNA methylation/demethylation with regulation by transcription factors. The mechanism is of interest not only because it achieves precise control of high levels of expression, but also because the targeted genes are predicted to modify cell walls during pollen tube growth. DNA segments containing promoters of some of these genes are sufficient to tie heterologous genes into this regulatory pathway in pollen, and we are exploring the potential of engineering methylation/demethylation both for experimental validation of pathway components and for synthetic biology.

Funding acknowledgement: National Science Foundation (NSF)

## P52

## Non-invasive identification and analysis of maize root exudates

(submitted by Caroline Henry <chenry@danforthcenter.org>)
Full Author List: Henry, Caroline[1]; Chavez, Leonardo[1]; Wei, Michael[1]; Duenwald, Joe[1]; Gutierrez, Katia[1]; Baldrich-González, Patricia[2]; Murphy, Katie[1]; Williams, Russell[1]
[1] Donald Danforth Plant Science Center
[2] University of California, Davis

Root exudates are a complex mixture of compounds secreted by plant roots into the soil. Although previous research has determined that root exudates have roles in plant-microbe interactions and responses to biotic and abiotic stress, much remains unknown about root exudates as they are difficult to capture. Most commonly, root exudates are collected from hydroponically grown plants. These experiments are not representative of field grown plants due to the absence of soil, and the vital plant-microbe interactions cannot be observed. In addition, removal of plants from their growth substrate does not allow for examination of root exudate composition at multiple time points in the plant's life cycle without serious disruption to natural root architecture. Thus, a less disruptive method of root exudate collection would contribute greatly to the discipline of plant-microbe interactions. Here we developed a non-invasive method for collection and analysis of metabolites and nucleic acids secreted by a plant into soil. Using this method, we observed significant differences between root exudate profiles from A) maize plants of different genotypes, and B) maize plants under abiotic stress (heat and/or drought).

Funding acknowledgement: Donald Danforth Plant Science Center

## P53

## Novel use of cell wall degrading enzymes as a tool to elucidate cell wall-mediated signaling during pathogenesis

(submitted by Megan DeTemple <mf6130@iastate.edu>)
Full Author List: DeTemple, Megan[1]; Frye, Emily[1]; Mugisha, Alither[1]; Swaminathan, Sivakumar[1]; Zabotina, Olga[1]
[1] Iowa State University; Ames; Iowa; 50011

The cell wall is the plant's first line of defense against microbial pathogens. This complex fabric of polysaccharides is not simply a passive barrier against the hundreds of cell wall-degrading enzymes (CWDEs) secreted by pathogens, but the modifications of the cell wall caused by their action can encode biological messages that allow the plant to perceive and respond to the pathogen. The long-term goal of the Zabotina lab is to understand the exact nature of these cell wall alterations and what plant immune responses are initiated. The lab uses the novel approach of expressing individual microbial CWDEs with a secretory peptide in Arabidopsis and most recently in Maize. Using this method, the plant cell wall is modified in very specific ways by individual CWDEs normally secreted by pathogens. The cell wall modifications in these transgenic plants caused by CWDEs were analyzed using biochemical methods and the plant's gene expression response was monitored via transcriptome analyses using RNA sequencing. Our results show that even small changes in the cell wall can upregulate plant defense genes in both Arabidopsis and Maize causing induced resistance against *Botrytis cinerea* and *Colletotrichum graminicola*. These results inform the importance of the cell wall in plant-pathogen interactions and indicate that the cell walls are intrinsically involved in plant immune responses to biotic stresses.

Funding acknowledgement: National Science Foundation (NSF), Iowa State University Crop Bioengineering Center

**P54** @lbriboldi

## Optimizing CRISPR-Cas9 genome editing for precise gene deletion in *Zea mays*

(submitted by Lucas Baiochi Riboldi <baiochir@msu.edu>)
Full Author List: Riboldi, Lucas B[1 2 3]; Gerasimova, Sophia V[2]; Pessoa, Mateus[2 3]; Lopes Filho, José H[2]; Pinto, Maísa S[2]; Bruno, Maria H F[2 3]; Nonato, Juliana V A[2]; Yassitepe, Juliana E C T[2 3 4]; Gerhardt, Isabel R[2 3 4]; Arruda, Paulo[2 3]; Dante, Ricardo A[2 3 4]
[1] Michigan State University, Plant Biology Department, East Lansing, United States of America
[2] Genomics for Climate Change Research Center (GCCRC), Universidade Estadual de Campinas (UNICAMP), Campinas, Brazil
[3] Universidade Estadual de Campinas (UNICAMP),Centro de Biologia Molecular e Engenharia Genética (CBMEG), Campinas, Brazil
[4] Embrapa Agricultura Digital, Campinas, Brazil

At the Genomics for Climate Change Research Center (GCCRC), we study genes of unknown function involved in drought response using genetic engineering approaches. We employ the CRISPR-Cas9 technology to induce precise deletions to inactivate gene expression. Six gRNAs targeting promoter and coding regions were designed to optimize deletion efficiency and were tested in protoplasts. To achieve the optimal frequency of deletion induction, the gRNAs were combined in pairs. Each pair of corresponding gRNA vectors was co-transformed into protoplasts with the vector containing Cas9 and nuclear-localized TdTomato reporter. The combination of gRNA1 and gRNA6 effectively excised the entire gene, validated by PCR and sequencing analyses, in two independent experiments. Both guides were cloned into a destination vector pG4GB411-BWM, which is compatible with the ternary vector system for Agrobacterium-mediated transformation and contains Cas9 under the control of the ZmUbi promoter, the morphogenic genes Wuschel (Wus) and *Babyboom* (Bbm), and the mCherry reporter. Plant transformation was carried out using B104 embryos. Genotyping confirmed complete deletions in 10 out of 100 T0 events. These plants were acclimated to controlled environments for one month and transferred to greenhouse conditions for further development. The edited plants successfully produced pollen and were used for crosses with B104 maize ears, generating viable seeds. PCR assays confirmed the absence of Cas9 in the edited plants, ensuring no transgene integration. Keywords: genome editing, protoplast transformation, gene deletion, maize.

Funding acknowledgement: FAPESP (Brazil), CNPq (Brazil)

## P55

# Overexpression of *PSTOL1-like* genes increases maize root surface area and biomass under low and high phosphorus conditions

(submitted by Sylvia Morais de Sousa Tinoco <sylvia.sousa@embrapa.br>)
Full Author List: Negri, Barbara F.[1]; Maciel, Laiane S.[2]; Coelho, Antonio M.[3]; Barros, Beatriz A.[3]; Guimarães, Claudia T.[1 2 3]; Magalhães, Sylvia M. S.[1 2 3]; Tinôco, Sylvia M. S.[1 3]
[1] Federal University of São João del-Rei (UFSJ), São João del-Rei, MG, Brazil, 36301-158
[2] Federal University of Minas Gerais (UFMG), Belo Horizonte, MG, Brazil, 31270-901
[3] Embrapa Maize and Sorghum, Sete Lagoas, MG, Brazil, 35701-970

Low phosphorus (P) availability in soil is a significant limitation for crop production in tropical regions. The PHOSPHORUS-STARVATION TOLERANCE1 (OsPSTOL1) protein kinase enhances root surface area, P acquisition, and grain yield in rice under P-deficient conditions. Homologs of OsPSTOL1 in sorghum were identified through association mapping in two sorghum panels phenotyped for P uptake, root system morphology, and architecture in hydroponic systems, as well as grain yield and biomass accumulation under low-P conditions in Brazil and Mali. In maize and sorghum, candidate genes were co-localized with quantitative trait loci (QTL) associated with root morphology, dry weight, and grain yield under low P. To validate the function of these genes, the rice *OsPSTOL*1 (as a positive control) and its maize (*ZmPSTOL*3.06, *ZmPSTOL*8.02, and *ZmPSTOL8.05*_1) and sorghum (*Sb07g002840, Sb03g031690*, and *Sb03g006765*) homologs were cloned downstream of an ubiquitin promoter in the pMCG1005 vector, with the *Bar* gene serving as a selective marker. Genetic transformation of maize B104 embryos was performed using *Agrobacterium tumefaciens*. Homozygous transgenic events with a single copy of the transgene were selected, and those showing transgene overexpression were evaluated under low and high-P conditions. In a growth chamber, the events *Sb07g002840*, *Sb03g006765*, and *ZmPSTOL3.06* showed greater root length and total fine root surface area compared to the negative control (B104 transformed with an empty vector), under both low and high P-conditions. The Sb03g006765 event exhibited higher root and shoot dry weight under both low and high P. *ZmPSTOL8.02* presented a higher dry weight than the negative control only under low P, while *Sb07g002840* had a higher shoot dry weight under high P. In the greenhouse, the *Sb03g006765*, *ZmPSTOL8.02*, and *ZmPSTOL8.05_1* events showed increased shoot dry weight under low P, while *OsPSTOL1*, *Sb07g002840*, and *Sb03g006765* exhibited higher shoot dry weight under high P. For root dry weight under low P, *Sb03g006765*, *ZmPSTOL8.02*, and *ZmPSTOL8.05_1* were superior to the negative control. Under high P, all events except *ZmPSTOL8.02* outperformed the negative control. Overexpression of the *PSTOL*1 homologs significantly improved vegetative growth and root surface area, demonstrating that these genes function similarly to *OsPSTOL*1 in rice.

Funding acknowledgement: Embrapa, GCP, Capes, CNPq

## P56

## Phenotypic characterization of overexpression of *glossy*3 involved in biosynthesis of cuticular wax in maize

(submitted by J.Aaron Avalos-Calleros <aaronac089@ksu.edu>)
Full Author List: Avalos-Calleros, J. Aaron[1]; Navrude, Spencer[1]; Liu, Hui[2]; Liu, Sanzhen[2]; Park, Sunghun[1]
[1] Kansas State University, Department of Horticulture & Natural Resources, Manhattan, Kansas, United States, 66506
[2] Kansas State University, Department of Plant Pathology, Manhattan, Kansas, United States, 66506

Cuticular wax in plants acts as a barrier against biotic and abiotic stresses, such as insects, fungal infections, and drought. Several *glossy* (*gl*) genes involved in cuticular wax biosynthesis have been identified through wax-deficient mutants exhibiting glossy leaf phenotypes. Our previous transient gene activation studies in maize cells have shown that the gene *glossy3* (*gl3*), which encodes a MYB transcription factor, upregulates at least 90 genes; nine of them are known *glossy* genes. This suggests that *gl3*-overexpressing maize may increase wax content, potentially enhancing tolerance to biotic and abiotic stresses. In this study, *gl3* driven by the rice ubiquitin (*OsUbi*) promoter was used to generate *gl3*-overexpressing maize lines. More than 15 transgenic plants were created using *Agrobacterium*-mediated immature embryo transformation with a previously established, simple, and efficient protocol that did not involve the use of any morphogenic genes. The transgenic lines were confirmed by their survival in a high-concentration bialaphos selection medium, *dsRed* reporter gene expression, and PCR analysis. Molecular and phenotypic characterization of *gl3*-overexpressing maize, including transgene expression, total wax content, biomass, yield, and plant height, will be presented in this poster.

Funding acknowledgement: United States Department of Agriculture (USDA)

## P57

## Red chlorophyll catabolite reductase mutants in maize and sorghum

(submitted by Jacob Olson <olson169@purdue.edu>)
Full Author List: Olson, Jacob[1 2]; Kaur, Amanpreet[1 2]; Khangura, Rajdeep[1 2]; Benke, Ryan[1 2]; Dilkes, Brian[1 2]
[1] Department of Biochemistry, Purdue University, West Lafayette, IN
[2] Center for Plant Biology, Purdue University, West Lafayette, IN

The porphyrin pathway is highly conserved and essential for all living organisms. Porphyrins are macrocyclic tetrapyrroles that are precursors to key compounds in electron transport, including chlorophyll and heme. Chlorophyll is photoreactive, absorbing photons and converting light energy to chemical energy. Multiple intermediates in the porphyrin and chlorophyll pathways are dangerously photoreactive, producing lethal quantities of free radicals and reactive oxygen species (ROS) when excited by light. Thus, the biosynthesis of these intermediates must be under strict control. Lesion-forming porphyrin catabolism mutants have been previously identified. These mutants are characterized by the formation of necrotic lesions in concentric circles that expand continuously covering the entire leaf and eventually the whole plant. Chlorophyll catabolism mutants *Accelerated cell death1, Lethal leaf spot1,* and *Dropdead1* are all encoded by orthologs of Pheophorbide A oxygenase in Arabidopsis, maize, and sorghum, respectively. *Accelerated cell death2* is encoded by red chlorophyll catabolite reductase in Arabidopsis, but no mutants in maize or sorghum are described. Red chlorophyll catabolite reductase acts on red chlorophyll catabolite, formed during the breakdown of chlorophyll, reducing the fluorescent red chlorophyll catabolite to a non-florescent chlorophyll catabolite, preventing the accumulation of phototoxic chlorophyll catabolites during leaf senescence. Here we describe red chlorophyll catabolite reductase mutants in maize and sorghum. The sorghum mutant, which we call *dropdead2 (ded2)*, is a deleterious missense EMS mutation. Co-segregation confirmed the mutation is required for lesion formation, and the segregation ratios are consistent with a recessive mutation. Co-segregation is currently being performed for maize mutants. We conducted light-dependent lesion formation experiments demonstrating the phototoxicity of our mutants. Finally, we profiled metabolites from our mutants to determine the abundance of chlorophyll and chlorophyll catabolites in our mutants compared to the wild type.

Gene / Gene Models described: *acd1, acd2, lls1, ded1*; Zm00001eb027950, SORBI_3003G146500
Funding acknowledgement: National Science Foundation (NSF), United States Department of Agriculture (USDA), Department of Energy (DOE)

## P58

## Restoration of haploid male fertility in maize: A novel approach using *Zmjr* mutants

(submitted by Md Nazmul Hossain <nhossain@iastate.edu>)
Full Author List: Hossain, Nazmul[1]; Pfeffer, Sarah[1]; Frei, Ursula K.[1]; Lübberstedt, Thomas[1]; Aboobucker, Siddique I.[1]
[1] Iowa State University, Ames, Iowa, USA 50010

Double haploid (DH) technology accelerates plant breeding by developing completely homozygous lines within two generations compared to six to eight generations in conventional breeding. Production of DH lines involves haploid induction, selection, and subsequent DH production. Haploid male and female fertility are a critical factor for successful DH production. Several plant species, including maize, exhibit haploid female fertility. However, haploid males, with one set of chromosomes, are mostly sterile due to unequal chromosomal distribution during meiosis I. Current practices rely extensively on colchicine-induced chromosome doubling to overcome haploid male sterility. Nevertheless, the underlying process is time-consuming, laborious, and inefficient. In our recent studies, we showed that mutation in either of the two genes in *Arabidopsis*; *AtPS1(parallel spindle 1)* or *AtJAS (Jason)* can facilitate the formation of parallel spindles instead of perpendicular spindles in meiosis II. This can enable the equal distribution of chromosomes in haploid meiosis II despite the erroneous meiosis I in haploids leading to the restoration of haploid male fertility (HMF) in *Arabidopsis*. However, the application of such genetic approaches to maize remains underexplored. Using bioinformatics approaches, we identified four *ZmJR* genes homologous to *AtJAS* in maize-*; ZmJR1 to ZmJR4*. These genes exhibit a similarity of 22% to 43% to AtJAS at the amino acid level. We have identified transposon insertion knockout maize mutants for these candidate genes. HMF restoration studies of these mutants are underway. Further, functional analysis of these candidate genes by complementation experiments is also in progress. This work is hoped to represent a significant leap forward in DH technology, with profound implications for accelerating plant breeding in maize and beyond.

Gene / Gene Models described: ; Zm00001d034278, Zm00001d013150, Zm00001d036637, Zm00001d053808
Funding acknowledgement: United States Department of Agriculture (USDA)

## P59

## Robust leaf-based transformation in *Sorghum bicolor* and editing of classical maize genes

(submitted by Max Braud <mbraud@danforthcenter.org>)
Full Author List: Braud, Max[1]; Wang, Zhonghui[1]; Yang, Bing[1,2]; Eveland, Andrea L[1]
[1] Donald Danforth Plant Science Center; St. Louis, MO, 63132
[2] University of MIssouri College of Agriculture, Food and Natural Resources; Columbia, MO, 65201

*Sorghum bicolor*, a water and nitrogen use efficient cereal crop that is closely related to maize, has recently gained notoriety as solutions to erratic environmental extremes are explored. Sorghum has multiple agronomic uses with varieties optimized for producing grain, sugar or cellulosic biomass, which makes it a prime candidate for food, feed and biofuel feedstock. Sorghum remains largely unimproved compared to maize in terms of yield. Although widely grown in arid regions of the world, the lack of yield improvement in sorghum has drawn less interest among U.S. farmers opting for more productive crops. Accelerating crop improvement in sorghum for sustainable agriculture demands broadening of available genetic tools and resources. Here, we optimized a sorghum transformation protocol using the grain accession Tx430 for use with embryonic or somatic-based explants. Our leaf-based transformation protocol with exogenously expressed morphogenic regulators *BBM and WUS* achieves modest transformation efficiency suitable for the generation of targeted gene edited lines using CRISPR/Cas9-based editing. Transgenic plantlets are moved to soil in less than 3 months with T1 seed harvested in about 6-7 months. As a proof of concept, we've generated loss-of-function mutations in the orthologs of genes underlying classical maize mutants including *liguleless1* and *teosinte branched1*. Both mutants in sorghum recapitulate phenotypes of their maize counterparts, though there are differences. We are continuing to edit associated regulatory elements to test mechanisms of functional regulation of these genes *in planta*.

Funding acknowledgement: Department of Energy (DOE)

## P60

## Roles of REL2 mediated transcriptional co-repression in maize immunity

(submitted by Brianna Griffin <bdg@iastate.edu>)
Full Author List: Griffin, Brianna D.[1]; Montes-Serey, Christian[1]; Galli, Mary[2]; Gallavotti, Andrea[2]; Walley, Justin W.[1]
[1] Department of Plant Pathology, Entomology, and Microbiology, Iowa State University, Ames, IA, USA, 50011
[2] Waksman Institute of Microbiology, Rutgers University, Piscataway, NJ, USA, 08854

Protein acetylation is a major post-translational modification that modulates many cellular processes, including plant immunity and stress responses. *Cochlibolus carbonum* (Northern Corn Leaf Spot) produces the effector HC-Toxin, a lysine deacetylase inhibitor required for pathogen virulence. RAMOSA1 ENHANCER LOCUS2 (REL2) is a transcriptional corepressor homologous to TOPLESS (TPL) in *Arabidopsis*. TPL family members are required for a range of biological processes, including development and immunity, and are critical components of hormone responses, including auxin and jasmonate signaling pathways. We identified a lysine acetylation site on REL2 using global acetylome profiling of maize treated with HC-Toxin or *C. carbonum.* Furthermore, we found that *rel2* loss of function mutant plants are susceptible to infection, demonstrating that REL2 is directly related to plant immunity. Using Yeast Two-Hybrid assays, we have shown REL2 that mimics acetylation results in reduced interaction of REL2 with transcription factors containing DLN and RLFGV repression motifs. We have further confirmed REL2 as a corepressor using luciferase corepression assays. Lastly, we have determined REL2-associated gene expression via transcriptomics. This work aims to elucidate how hyperacetylation impacts the biological activity of REL2 and REL2's roles in plant-pathogen interactions. These results will be utilized to gain a more detailed molecular understanding of plant immunity and to reconstruct a model for how REL2 transcriptionally regulates plant pathogen response.

Gene / Gene Models described: *REL2*; GRMZM2G042992
Funding acknowledgement: United States Department of Agriculture (USDA)

## P61

## Sorbitol dehydrogenase enhances kernel size by integrating carbohydrate and redox metabolism in the hypoxic endosperm

(submitted by Nadia Mourad <nmourad@ufl.edu>)
Full Author List: Mourad, Nadia[1]; Hilo, Alexander[2]; Rolletschek, Hardy[2]; Borisjuk, Ljudmilla[2]; Guan, Jiahn-Chou[1]; Koch, Karen E.[1]
[1] Plant Molecular and Cellular Biology Program, Horticultural Sciences Dept., University of Florida, Gainesville, FL
[2] Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany

During grain fill, sorbitol accumulates in the maize endosperm, a hypoxic microenvironment. Unlike other plant systems, sorbitol biosynthesis in maize kernels is not driven by desiccation and declines as kernels mature. Sorbitol dehydrogenase (SDH) catalyzes the only known path for interconversion of (fructose + NADH) ↔ (sorbitol + NAD+) in the endosperm. To evaluate the role of sorbitol, we characterized an *Ac/Ds*-induced *sdh1* mutant with undetectable SDH activity and trace sorbitol levels in kernels. The dry weight of s*dh1* mutant kernels is about 13–17% less than that of wild type at maturity, corresponding to a reduced rate of starch accumulation.Metabolite analyses of *sdh1* kernels revealed that levels of soluble sugars, including fructose (~40%) and sucrose (30–39%), are significantly elevated. Notably, the level of NADH, one substrate for the SDH enzyme reaction, rises in mutant kernels, indicating a redox imbalance in the endosperm. Sorbitol is most abundant in the central endosperm where oxygen is least available, supporting a potential role for SDH in maintaining redox balance in this low-oxygen organ. These results suggest two potential functions of SDH in kernel development: 1) SDH-mediated conversion of fructose to sorbitol facilitating sucrose import by promoting its rapid metabolism, and thus also starch synthesis, and 2) SDH activity replenishing $NAD^+$ needed to maintain glycolytic flux in the hypoxic endosperm.Findings underscore regulatory contributions by SDH in optimizing grain size and starch storage through integration of carbohydrate and redox metabolism.

Gene / Gene Models described: *sdh1*; Zm00001d031727
Funding acknowledgement: National Science Foundation (NSF)

## P62

### Systematic exploration of transcription factor function in maize

(submitted by Taylor Scroggs <taylor.scroggs@uga.edu>)
Full Author List: Scroggs, Taylor[1]; Nelms, Brad[1]
[1] University of Georgia, Athens, Ga, United States, 30605

Transcription factors (TFs) have various functions in plant development. In a model crop like Maize, there are thousands of TFs and due to the sheer amount of them, we don't know the function of many. I have developed a system to systematically explore TF function with the use of high throughput ectopic expression of individual TFs. Ectopic expression of TFs induces the native pathways and with that information, we can begin to tease apart the native function of those TFs. We have established methods for high-throughput transformation of leaf protoplasts in 384-well plates. Median transformation efficiencies are >70% and consistent between days and across the plates. We have used this to express 164 individual TFs in duplicate and measure the full transcriptome-wide response by RNA sequencing. Replicate samples consistently show a higher Pearson correlation than random sample pairs. 30% of TFs cluster immediately next to their replicate, indicating that many TFs induce reproducible responses and the responses are sufficiently distinct to drive clustering. We also find evidence supporting that at least some of the genes induced are likely direct TF targets. For example, the knotted1 (kn1) motif is enriched in the promoters of genes induced by kn1 overexpression. In the next steps, we will continue the established workflow through the remainder of the cloned maize TFome, producing a resource for the plant community.

Funding acknowledgement: National Science Foundation (NSF)

## P63

### Targeted seed EMS mutagenesis reveals a bHLH transcription factor underlying male sterility in sorghum

(submitted by Yuguo Xiao <yxiao@danforthcenter.org>)
Full Author List: Xiao, Yuguo[1]; Khangura, Rajdeep S.[2]; Wang, Zhonghui[1]; Dilkes, Brian P.[2]; Eveland, Andrea L.[1]
[1] Donald Danforth Plant Science Center, St. Louis, MO
[2] Purdue University, West Lafayette, IN

Forward genetic screens of mutant populations are essential for functional genomics studies and requires isolating independent mutant alleles to molecularly identify causal genes. Recovering multiple alleles is challenging in many species. Here, we demonstrate how classic seed EMS mutagenesis coupled with sector analysis and whole-genome sequencing can overcome this limitation by validating a causal locus for the *male sterile 8* (*ms8*) mutant in sorghum. We identified the *MS8* gene as *Sobic.004G270900*, encoding the sorghum ortholog of maize *bhlh122*, a transcription factor essential for male fertility in maize. Bulked segregant analysis mapped *ms8-1* to a region on chromosome 4 containing *Sobic.004G270900*. Seeds from heterozygous *MS8*/*ms8-1* plants were mutagenized and screened for chimeric inflorescences containing sectors with white, sterile anthers resembling the *ms8-1* homozygous phenotype. Genome sequencing of sterile and fertile sectors from a single chimeric inflorescence revealed two mutations in *Sobic.004G270900* within the sterile sector, but not the fertile sector. Isolation of this loss-of-function allele (*ms8-2*) established *Sobic.004G270900* as the causative locus for male sterility in the *ms8* mutant. To further validate this finding, we leveraged an in-house leaf-based sorghum transformation and CRISPR/Cas9-based gene editing pipeline to edit the *MS8* locus in a different genetic background. In addition to generating several novel alleles of *ms8* for genetic studies in sorghum, we demonstrate a novel and straightforward genetic tool for researchers who lack access to advanced transformation facilities to validate gene candidates. Unlike gene editing approaches, targeted seed EMS mutagenesis requires no prior knowledge of candidate genes, providing an unbiased method for pinpointing causal loci and advancing functional genomics research.

Gene / Gene Models described: *MS8*; Sobic.004G270900
Funding acknowledgement: United States Department of Agriculture (USDA), Department of Energy (DOE)

**P64** @mattdhelm19

## The maize tar spot pathogen *Phyllachora maydis* encodes an effector protein that targets the chloroplasts and suppresses plant immunity

(submitted by Matthew Helm <Matthew.Helm@usda.gov>)
Full Author List: Cameron, Terri[1]; Jaiswal, Namrata[1]; Rogers, Abigail[2]; Iyer-Pascuzzi, Anjali[2]; Kim, Saet-Byul[3]; Helm, Matthew[1]
[1] Crop Production and Pest Control Research Unit, U.S. Department of Agriculture-Agricultural Research Service (USDA-ARS), West Lafayette, IN 47907, U.S.A.
[2] Department of Botany and Plant Pathology, Purdue University West Lafayette, IN 47907, U.S.A.
[3] Department of Plant Pathology and Center for Plant Science Innovation, University of Nebraska-Lincoln, Lincoln, NE 68583, U.S.A.

Chloroplasts are essential organelles that have a foundational role during molecular plant-pathogen interactions by regulating the biosynthesis and production of key defense-related phytohormones during pathogen infection. As such, fungal pathogens have evolved virulence proteins known as effectors that strategically target and interfere with chloroplast functions, leading to a disruption of chloroplast-derived immune responses, compromised photosynthesis, and accelerated disease development. One such fungal pathogen that uses effector proteins during host infection is *Phyllachora maydis* (*P. maydis*), a foliar pathogen of maize that causes tar spot disease. Despite its importance to U.S. agriculture, our knowledge regarding whether this fungal pathogen uses effector proteins to target chloroplasts remains limited. In this study, we leveraged the availability of a *P. maydis* genome and accompanying transcriptome to select fungal effector proteins that encode predicted chloroplast targeting peptide sequences and which are expressed during the infection process. In total, we identified fifteen effector proteins from *P. maydis* that met our stringent selection criteria. To confirm if the *P. maydis* effectors localize to chloroplasts, we fused each of the fungal effectors to super Yellow Fluorescent Protein (sYFP) and transiently expressed the effector-fluorescent protein fusions in the model plant *Nicotiana benthamiana*. Laser-scanning confocal microscopy revealed that of the fifteen fungal effectors, one fungal effector consistently targeted the chloroplasts, which we have designated PmEC2851. Importantly, we show the chloroplast targeting peptide sequence from PmEC2851 is necessary and sufficient for accumulation in the stroma of chloroplasts and that PmEC2851 attenuates multiple host immune responses. Collectively, our results provide valuable insights into the putative function of a chloroplast-localized effector protein from *P. maydis* and will likely stimulate new research aimed at elucidating the molecular mechanisms potentially manipulated by this fungal pathogen.

Funding acknowledgement: United States Department of Agriculture (USDA)

## P65

## The tale of two rubisco activase: studying the function of *rca1* and *rca3* in maize.

(submitted by Mae Mercado <mercado6@illinois.edu>)
Full Author List: Mercado, Mae Antonette[1]; Om, Kuenzang[2]; Cousins, Asaph[2]; Studer, Anthony[1]
[1] Department of Crop Sciences, University of Illinois Urbana-Champaign
[2] School of Biological Sciences, Washington State University

RUBSICO activase (RCA) is a protein that removes inhibitory sugar phosphates bound to ribulose-1,5-bisphosphate carboxylase/oxygenase (RUBISCO) to do carboxylation. In maize, there are two isoforms of *rca*: the α isoform, RUBISCO activase 3 (*rca3*, Zm00001eb164380), and the β isoform, RUBISCO activase 1 (*rca1*, Zm00001eb164390). Between these two isoforms, *rca1* is expressed in maize constitutively, while *rca3* is hypothesized to be expressed only when plants are exposed to heat stress. The function of the α isoform has been well-studied in $C_3$ species but not in maize or other $C_4$ plants. In this study, we show that *rca3* is thermally induced, with levels significantly upregulated after four hours at 42°C. A *Dissociation* transposon insertion in the second exon of *rca3* created a complete knockout of *rca3*, and plants homozygous for this mutation showed decreased net $CO_2$ assimilation in mutant plants compared to wildtype plants after heat treatment. In terms of RUBISCO content of wildtype and mutant plants, there was a significant decrease in the amount of RUBISCO for both wildtype and mutant plants four hours after heat stress exposure, which indicates that the mutation in *rca3* is not affecting the amount of RUBISCO but potentially affecting the amount of active RUBISCO or the number of active sites. To investigate the β isoform, a *Dissociation* transposon tagging screen was performed, which identified an insertion in exon 3 of *rca1*. Interestingly, homozygous individuals were seedling lethal, indicating its importance in maize development and survival. Further investigation of the *rca1* mutant under heat stress will reveal whether *rca3* can rescue the lethal phenotype upon heat induction.

Gene / Gene Models described: *rca1*, *rca3*; Zm00001eb164390, Zm00001eb164380
Funding acknowledgement: Department of Energy (DOE), Illinois Corn Growers Association

## P66

## Transcriptomic analysis of tar spot and fisheye lesions exhibit distinct yet overlapping transcriptomic signatures, suggesting a complex interplay of shared and specific pathways

(submitted by Raksha Singh <rakshya.singh@gmail.com>)
Full Author List: Singh, Raksha[1]; Gómez Gutiérrez, Sandra[2]; Gribskov, Michael[2]; Goodwin, Stephen[1]
[1] USDA-ARS
[2] Purdue University

*Phyllachora maydis*, an obligate fungal pathogen, causes tar spot, a severe maize foliar disease impacting global corn production. Tar spot symptoms appear as small, raised, irregularly shaped black lesions called stromata scattered on leaves, stalks, and husks. In severe cases, stromata can be surrounded by brown, elliptical necrotic halos known as fisheye lesions inducing extensive necrosis, premature senescence, and plant death. Fisheye lesions have been previously linked with *Microdochium maydis* in Mexico, but its presence in the United States has not yet been confirmed. The molecular basis of pathogenicity of *P. maydis* resulting in tar spot and fisheye symptoms remains poorly understood. Here, we used RNA sequencing (RNA-Seq) of tar spot stromata with and without fisheye lesions to compare their transcriptomes and identify genes and pathways associated with each symptom. Comparative analysis revealed distinct and significantly differentially expressed gene patterns (DEGs) associated with each symptom. Notably, reads from *P. maydis* were dominant in both samples, alongside unknown taxa from suborders *Masssarineae* and *Pleosporinae*, suggesting the presence of additional unidentified species. Benchmarking Universal Single-Copy Orthologs (BUSCO) analysis further revealed that *P. maydis* was 92.6% and 92.0% complete in both samples, while *Massarineae* was 58.5% and 83.7% complete and *Pleosporinae* was only 4% and 8.9% complete. These findings suggest the existence of new, unknown taxa associated with tar spot with fisheye lesions and highlight both common and distinct genes and pathways associated with tar spot with and without fisheye lesions. Further exploration of the identified findings could lead to valuable insights into *P. maydis* pathogenicity and potentially contribute to the development of targeted disease control strategies.

Funding acknowledgement: United States Department of Agriculture (USDA)

## P67

## Transcriptomic and metabolomic analyses of oxylipin-deficient maize mutants

(submitted by Charles Hunter <charles.hunter@usda.gov>)
Full Author List: Hunter, Charles T[1]; Gorman, Zachary[1]; Hayford, Rita K[2]; Li, Qin Bao[1]; Andorf, Carson M[2 3]; Christensen, Shawn[4]; Block, Anna[1]; Woodhouse, Margaret R[2]
[1] USDA-ARS Chemistry Research Unit, Gainesville, FL 32608, USA
[2] USDA-ARS Corn Insects and Crop Genetics Research Unit, Ames, IA 50011, USA
[3] Department of Computer Science, Iowa State University, Ames, IA, 50011, USA
[4] Nutrition, Dietetics & Food Science, Brigham Young University, Provo, UT 84602, USA

The lipoxygenase (LOX) pathway produces several oxylipins that regulate plant defense against insect herbivores, including the phytohormone jasmonic acid (JA). To gain a better understanding of the impact of JA and other oxylipins on maize defense response to herbivory by fall armyworm (FAW), we performed transcriptomic and metabolomic analyses on two CRIPSR-induced mutants in the oxylipin pathway, lipoxygenase 10 (lox10) and allene oxide cyclase 1 and 2 (aoc1/2), both of which are highly susceptible to FAW. Targeted metabolomic analysis show reductions in most 13-oxylipin products in both mutants, yet they differentially accumulate herbivory-induced jasmonic acid (JA), but not its precursor, 12-OPDA. Despite producing similar levels of 12-OPDA, aoc1/2 mutants produce almost no JA in response to herbivory while lox10 produces around 50% of wildtype levels. Comparison of aoc1/2 and lox10 in the same genetic background provides an opportunity to differentiate the roles of JA and other 13-LOX derived oxylipins. An expanded untargeted metabolomics approach to compare the FAW-induced responses of these mutants showed approximately 7,500 metabolic features between FAW-treated and untreated plants in WT, aoc1/2, and lox10 genotypes. Substantial differences in metabolic responses to FAW were seen in both mutants, though this was more dramatic in the JA-deficient aoc1/2 than in lox10 mutants. Transcriptomic analyses (RNAseq) of these same tissues revealed major impacts of herbivory on gene expression in both mutants, though again, a greater affect was seen in aoc1/2 than in lox10 mutants. Our work provides a rich source of metabolomic and transcriptomic data on maize responses to FAW herbivory and helps to distinguish the impact of LOX10-derived oxylipins from JA. Additionally, this can help to differentiate JA-dependent vs. independent defenses, and possibly help identify novel JA-independent sources of FAW resistance in maize.

Gene / Gene Models described: *lox10*, *aoc1*, *aoc2*; Zm00001d053675, Zm00001d029594, Zm00001d047340
Funding acknowledgement: United States Department of Agriculture (USDA)

## P68

## Tropical maize: paving the way to explore untapped genetic diversity

(submitted by Alessandra Koltun <alessandra.koltun@corteva.com>)
Full Author List: Koltun, Alessandra[1]; Wang, Ning[1]; Gao, Huirong[1]; Lenderts, Brian[1]; McDaniel, Mark[1]; Tice, Lauren[1]; Gordon-Kamm, William[1]
[1] Corteva Agriscience

**Abstract**: Maize (*Zea mays*) is a vital crop with global nutritional, economic, and cultural significance, and serves as a valuable genetic model. Despite the rich genetic diversity in tropical maize lines, their potential for crop improvement remains largely untapped. This study evaluates the efficiency of genetic transformation and genome editing in tropical maize using leaf explants and morphogenic genes. Four tropical lines (TL) were transformed with vectors containing the transcriptional regulators Baby Boom and Wuschel in different configurations or without them (control). To assess genome editing efficiency, a CRISPR-Cas9 system targeting granule-bound starch synthase-I (GBSS), encoded by *WAXY1*, was employed. The control experiment without morphogenic genes yielded no transformants. Among eight constructs with transcriptional regulators, Construct 6 yielded the highest transformation efficiency for TL1 (353%) and TL2 (440%). In a second screening, Construct 4 outperformed Construct 2 in all four genotypes. Small- and large-scale experiments showed comparable transformation and excision rates, though single copy, quality event (QE), and usable QE frequencies varied due to differences in copy number and T-DNA insertion patterns. For *WAXY1* gene editing, TL3 showed the highest editing efficiency (89.6%). Among three guide RNAs tested, gRNA-C had the highest efficiency, with a mutation rate of 92.7%, while gRNA-A showed lower efficiency at 73.3%. Dropout rates were higher between gRNA-B and gRNA-C (9.05%). Most mutations were biallelic (81.72%), with 18.28% monoallelic. These findings demonstrate that tropical maize can be efficiently transformed and edited, enhancing breeding programs for both tropical and temperate maize. **Keywords**: Baby boom, Wuschel, leaf whorls, genome editing.

# P69

## Understanding anthocyanin accumulation in developing pericarp tissue through comparative transcriptomics of near-isogenic and inbred purple maize cultivars

(submitted by Holly Anderson <hollyaa2@illinois.edu>)
Full Author List: Anderson, Holly[1]; Paulsmeyer, Michael[2]; Juvik, John[1]
[1] Department of Crop Sciences, University of Illinois, Urbana-Champaign, Urbana, IL 61801, USA
[2] Vegetable Crops Research Unit, USDA-ARS, Madison, WI, 53706, USA

Anthocyanins are plant-derived pigments of interest to the food industry as natural colorants. While anthocyanin biosynthesis has been well-documented in the maize aleurone layer, little is known about the metabolic processes of the pericarp layer. Using pericarp-pigmented maize, we performed RNA-seq, differential gene expression analysis, and Ultra High-Performance Liquid Chromatography to characterize anthocyanin biosynthesis in maize pericarp. Two experimental designs were performed: (1) a time-series analysis of whole pericarp tissue from three pigmented lines (B73 Color Converted, Amazonas, and Maize Morado) and an unpigmented control (B73) at 10, 15, and 20 days after pollination (DAP), and (2) a comparison of pigmented and unpigmented pericarp tissue fractions from four pigmented lines (B73 Color Converted, Mo17 Color Converted, Amazonas, and Maize Morado) harvested at 18 DAP. Significant expression of regulatory genes, *Pl1* (*Purple plant 1*) and *Lc1* (*Leaf color1*), encoding the predicted R2R3-MYB and bHLH proteins, respectively, was found across both experiments. Two genes, the canonical transporter *Bz2* (*Bronze2*) and *Wrky33*, a candidate regulatory gene, exhibited consistently high expression at 18 DAP and were correlated with anthocyanin content. Additional candidate genes included an alternative to the canonical *Bz1* (*Bronze1*) gene, *Ugt1* (*UDP-glucosyl transferase1*), a multidrug and toxic compound extrusion (MATE) transporter gene (*Mate12*), and a gene proposed to function in anthocyanin degradation (*Glu9* [*beta-glucosidase9*]). Samtools variant calling was performed on the purple near-isogenic lines and compared to B73 to find fixed loci associated with pericarp pigmentation. Major regulatory genes *P1*, *Pl1*, and *Lc1* were fixed, implicating their role in increasing anthocyanin content. This study supports the role of canonical anthocyanin biosynthetic structural genes and an analogous MBW complex in pericarp pigmentation, while also identifying novel candidate genes that may influence anthocyanin content. Further research is needed to validate the role of candidate regulatory and structural genes to enhance marker-assisted selection for anthocyanin content.

Funding acknowledgement: IL Corn Growers Association

## P70

## Understanding the role of TOR signaling and translational machinery in regulating protein-bound amino acid homeostasis in maize kernels

(submitted by Huda Ansaf <hah34@umsystem.edu>)
Full Author List: Ansaf, Huda[1]; Shrestha, Vivek[2]; Yobi, Abou[1]; Angelovici, Ruthie[1]
[1] Division of Biological Sciences and Interdisciplinary Plant Group, University of Missouri, Columbia, MO, 65211, USA
[2] Bayer Crop Sciences, St. Louis, Missouri, United States 63167

Cereal grains are the lifeline of the world's food supply. However, many cereal crops are not a complete source of dietary protein as they lack sufficient essential amino acids (EAAs), vital for human health and development, as well as monogastric livestock. The low protein quality in cereals is mainly due to the prevalence of seed storage proteins (SSPs), which are inherently deficient in certain EAAs. Interestingly, despite significant genetic perturbations in SSP content, the overall amino acid composition often remains unchanged due to proteomic rebalancing. This natural phenomenon poses a major challenge for biofortification efforts. To explore the molecular mechanisms regulating protein-bound amino acids (PBAAs), we performed comparative developmental proteomics on wild-type and opaque-2 knockout mutant maize kernels. The opaque-2 mutation, which disrupts the bZIP transcription factor, significantly reduces 22 kDa $\alpha$-zein levels while maintaining a similar PBAA composition to the wild type, with slightly elevated lysine content. Proteomics and polysome profiling revealed mis-regulation of translational machinery components and differences in polysome profiles across seed maturation. Phosphoproteomics analysis revealed altered phosphorylation of substrates regulated by the master regulator, Target of Rapamycin (TOR), and its interactors. As the TOR signaling pathway is integral to translational control, these findings point to a critical role for TOR and translational regulation in maintaining PBAA homeostasis in seeds. In addition, a candidate gene genome association approach identified high-confidence genes within the TOR pathway and those linked to translational machinery. Targeting the TOR signaling pathway could offer a promising avenue for genetic and agronomic strategies aimed at enhancing the nutritional quality of maize by optimizing seed protein content and composition.

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## P71

## What's new at the Maize Genetic Cooperation Stock Center

(submitted by Jeffery Gustin <jeff.gustin@usda.gov>)
Full Author List: Gustin, Jeffery[1]; Zimmerman, Shane[1]; Sachs, Marty[1]
[1] USDA-ARS Maize Genetics Cooperaion Stock Center, Urbana, IL 61801

The Maize Stock Center provided the maize genetics community with genetic stocks for nearly a century. During that time, the collection has grown to contain over 42,000 stocks, created by our community and donated to the Stock Center for storage, maintenance and distribution. The following are new or continuing initiatives at the Stock Center to increase the access and utility of the collection to the maize genetics community.**New Stock Images** – We've begun collecting standardized images of all mutant stocks at relevant growth stages including flatbed scans of kernel and cob mutants, sand bed images of seedling mutants and field images of later stage mutants. These are particularly useful for the large number of mutants that have not yet been genetically characterized. The images are currently being used by Stock Center Staff to improve efficiency and ensure line purity, but we plan to make them accessible to the public through MaizeGDB stock record pages.**New Allele Tests** – The Stock Center contains over 3400 uncharacterized 'phenotype-only' mutants. We continue to conduct allele tests between these mutants and well characterized mapped mutants with similar phenotypes. We have focused recently on short stature mutants such as dwarf, brachytic, and nana and have identified novel short stature mutants that are not allelic to mapped mutants as well as alleles of characterized mutants such *brachytic2*.New BSASeq Pipeline – Advances in short read sequencing have reduced the cost of mapping mutants using Bulk Segregant Analysis sequencing (BSAseq) to a price point that is on par with the cost of traditional field-based methods. With the guiding assistance of publications from Maize scientists, we have implemented a BSAseq pipeline to map phenotype-only mutants for under $200. We are using BSAseq along with traditional allele tests to add genomic position information to the collection of phenotype-only mutants.

Funding acknowledgement: United States Department of Agriculture (USDA)

**P72** @Mike82760453

## ZmCER9-mediated regulation of autoactive NLR proteins and effector-triggered immunity via ERAD pathway

(submitted by Wen-Yu Liu <wliu34@ncsu.edu>)
Full Author List: Liu, Wen-Yu[1]; Karre, Shailesh[2]; Long, Terri[1]; Balint-Kurti, Peter[2 3]
[1] Department of Plant and Microbial Biology, NC State University, Raleigh, USA
[2] Department of Entomology and Plant Pathology, NC State University, Raleigh, USA
[3] Plant Science Research Unit USDA-ARS, NC State University, Raleigh, USA

Nucleotide-binding leucine-rich repeat (NLR) resistance proteins play critical roles in plant defense by triggering effector-triggered immunity (ETI) upon pathogen effector recognition. However, inappropriate activation of ETI, often accompanied by hypersensitive response (HR), can be detrimental to plant growth. Rp1-D21, an autoactive derivative of the maize NLR Rp1-D, spontaneously induces HR, providing a model to study ETI regulation. Through genome-wide association mapping, we identified ZmCER9, a maize E3 ligase homologous to the Doa10 family, as a key modifier of the Rp1-D21 HR phenotype. ZmCER9 is an active E3 ligase localized to the endoplasmic reticulum (ER), where it mediates the proteasome-dependent degradation of Rp1-D21 and other autoactive NLR derivatives, but not their non-autoactive counterparts. The *ZmCER9* homolog in Arabidopsis is known as *AtCER9* has been previously associated with cuticle formation and drought stress. We observed that Arabidopsis *Atcer9* knockout mutants display enhanced HR, relative to wild type, when infected with pathogens that induce ETI . When ZmCER9 is overexpressed in these *Atcer9* mutants, HR is suppressed. We suggest therefore that this study describes a conserved, previously uncharacterized mechanism in plants in which CER9 directs the degradation of activated NLRs, maintaining immune homeostasis and preventing detrimental ETI overactivation.

Gene / Gene Models described: *ZmCER9*; Zm00001eb387290
Funding acknowledgement: National Science Foundation (NSF), United States Department of Agriculture (USDA)

## P73

## k1C resiliance: 400kb deletion of alpha kafirin family genes yields negligible nonkafirin proteome compensation in sorghum

(submitted by Tyler Ferris <tyler.ferris@huskers.unl.edu>)
Full Author List: Ferris, Tyler W[1]; Hurst, Preston J[1]; Altartouri, Bara[2]; Rose, Devin[3]; Li, Axia[1]; Clemente, Thomas[1]; Holding, David R[1]
[1] Center for Plant Science Innovation; Department of Agronomy and Horticulture; University of Nebraska-Lincoln; Lincoln, Nebraska, 68588
[2] Morrison Microscopy Core Research Facility; Nebraska Center for Biotechnology; University of Nebraska-Lincoln; Lincoln, Nebraska, 68588
[3] Food Innovation Center; Department of Food Science and Technology; University of Nebraska-Lincoln; Lincoln, Nebraska 68588

Grain sorghum (Sorghum bicolor (L.) Monech) is a crop of tremendous significance. Grain sorghum is cultivated for consumption by humans and livestock alike worldwide and is valued for its resistance to biotic and abiotic stresses. However, sorghum grain protein is deficient in essential amino acids and has low digestibility. Furthermore, sorghum does not yield flour with desirable bread making properties. These nutritional shortcomings can be attributed to the structure and amino acid content of the kafirin storage proteins that constitute >70% of proteins expressed in the endosperm that form low-digestibility protein bodies. This study was conducted to evaluate nutritional and biochemical characteristics of reduced kafirin, low-amylose sorghum grain. Previously, a single-guide RNA (sgRNA) Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR/Cas9) construct was used to target members of the alpha-kafirin gene family, k1C, which reduced kafirin expression in endosperm cells and elicited a proteome re-balance wherein the increase in nonkafirin expression and reduction in protein body morphology would increase lysine and improve digestibility of the grain. Additionally, introgression of the waxya mutant into k1C-edited F1 sorghum was performed to confer the low-amylose starch trait to improve the dough-making potential of sorgum grain. However, in these lines we see a lack of proteome rebalancing in favor of nonkafirin expression in spite of a ~400kb genomic deletion within the k1C family resulting in 12 active genes deleted. Biophysical results supporting this claim include transmission electron microscopy (TEM) images of protein bodies, dissecting microscope imagery of endosperm texture, protein-bound and free amino acid profile, and kafirin and non-kafirin sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS PAGE). The results of these tests provide insight into the resiliance of the k1C family's expression as well as the challenges implementing CRISPR transformation in highly-repetative portions of the genome for eliciting high-digestibility, high-lysine grain in sorghum and related species.

Funding acknowledgement: United States Department of Agriculture (USDA)

# P74

## A ZmWUSCHEL1-LITTLE ZIPPER-ROLLED LEAF1/REVOLUTA regulatory module controls stem cell organization in maize inflorescences

(submitted by Zongliang Chen <zc176@rutgers.edu>)
Full Author List: Chen, Zongliang[1]; Galli, Mary[1]; Strable, Josh[3]; Bang, Sohyun[4]; Schmitz, Robert[4]; Gallavotti, Andrea[1 2]
[1] Waksman Institute of Microbiology, Rutgers University, Piscataway, NJ, USA
[2] Department of Plant Biology, Rutgers University, New Brunswick, NJ, USA
[3] Department of Genetics, Development and Cell Biology, Iowa State University, Ames, IA USA
[4] Department of Genetics, University of Georgia Athens, GA, USA

Inflorescence meristems (IMs) balance stem cell proliferation mainly through CLAVATA-WUSCHEL feedback signaling between stem cells at the meristem tip and the underlying organizing center. We have identified a novel regulatory module composed of ZmWUSCHEL1 (ZmWUS1), LITTLE ZIPPER (ZPR), and ROLLED LEAF1/REVOLUTA (RLD1/REV) that directly balances stem cell proliferation and differentiation within the rib domain that subtends the organizing center. Previously, we demonstrated that maize dominant mutant *Barren inflorescence3* (*Bif3*) overexpresses *ZmWUS1* transcripts in an unusual ring-shaped pattern in ear primordia due to a drastic functional domain rearrangement in IMs. Overexpression of a duplicated copy of *ZmWUS1* driven by a cytokinin-hypersensitive promoter underlies the *Bif3* phenotype. Here, we show that in *Bif3*, ZmWUS1 modulates the expression of rib-domain expressed genes *ZmZPR4a* and *ZmZPR4b* in a mirroring ring-shaped pattern. Single-cell ATAC-seq analysis revealed that *ZmZPR4b* has a more accessible proximal promoter region in *ZmWUS1*-expressing cells of *Bif3* ear primordia compared to wild-type ears and is overexpressed in *Bif3* ear primordia. This suggests that in *Bif3* ear primordia, *ZmZPR4a*/*b* upregulation partially underlies the IM's rearrangement. Yeast two-hybrid assays, AlphaFold structure prediction, and single and double DAP-seq experiments indicate that ZmZPR4a/b, along with their closest paralog, ZmZPR3, physically interact with RLD1 via their bZIP domains. This interaction prevents RLD1 homodimerization, thereby inhibiting its DNA binding activity. CRISPR-Cas9-mediated triple knockout of *ZmZPR3*/*ZmZPR4a*/*b* yielded no obvious ear defects. However, CRISPR-Cas9-mediated triple knockout of *RLD1* and its closest paralogs, *RLD2* and *RLD3*, produced *Bif3-like* ear defects, including shortened ears with primordia lacking an obvious IM. Together with their overlapping expression patterns, these findings support a model where upregulation of *ZmWUS1* in *Bif3* ears prevents RLD1's requisite physical interaction with inhibitory ZPR proteins in the IM, revealing a previously unknown ZmWUS1-ZPR-RLD1 regulatory module controlling stem cell organization in maize inflorescences.

Funding acknowledgement: National Science Foundation (NSF)

**P75** @Xiaosa_Xu

## A high-resolution, meristem stage-specific single-cell gene expression atlas resolving developmental dynamics in maize inflorescence architecture

(submitted by Xiaosa Xu <xjkxu@ucdavis.edu>)
Full Author List: Xu, Xiaosa[1 2]; Passalacqua, Michael[2]; Gillis, Jesse[2 3]; Jackson, David[2]
[1] Department of Plant Biology, University of California, Davis, CA 95616, USA
[2] Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724, USA
[3] Department of Physiology, University of Toronto, Toronto, Ontario, M5S 3E1, CANADA

Maize productivity depends on the precise regulation of inflorescence development, which involves a series of programmed meristem fate transitions and intricate communication between diverse cell populations. However, our understanding of these processes has been limited by genetic redundancy, pleiotropy, and the morphological complexity of maize meristems. Our previous work using single-cell RNA sequencing (scRNA-seq) on whole ear primordia (Xu et al., 2021, *Developmental Cell*) provided an overview of cell types and meristem domains but lacked the resolution necessary to capture dynamic transitions between distinct meristem types. To address this limitation, we developed a high-resolution, stage-specific scRNA-seq atlas of developing maize ear inflorescence through precise dissection of key developmental regions. We generated scRNA-seq datasets for four major stages of ear meristem development: inflorescence meristem/early spikelet pair meristem, late spikelet pair meristem, spikelet meristem, and floral meristem. Cluster annotation revealed conserved cell types and spatial domains across stages, while differential expression analysis uncovered dynamic gene expression patterns critical for meristem fate transitions. In parallel, we constructed a high-resolution scRNA-seq atlas of developing tassel inflorescences. Tassels, unlike ears, form branches, and through fine dissection and single-cell profiling of tassel branch primordia, we found that their cell type composition closely resembles that of maize ear tips. This provides valuable insights into conserved and divergent cellular programs underlying the architectural differences between tassels and ears. Finally, we identified candidate genes with dynamic expression across meristem stages and employed CRISPR-based functional analysis to characterize their roles. High-order CRISPR mutants are being generated to further reveal key regulatory networks controlling maize inflorescence architecture. This work establishes the most comprehensive single-cell gene expression dataset of maize inflorescence development to date, offering unprecedented insight into the genetic regulation of meristem identity and transitions. These findings provide a powerful resource for maize research and breeding strategies targeting improved grain yield and architecture.
NSF, UC Davis new faculty startup and Agricultural Experiment Station.

Funding acknowledgement: National Science Foundation (NSF)

**P76** @Xiaosa_Xu

## A nuclear role of RAMOSA3 in inflorescence branching independent of its enzymatic function

(submitted by Xiaosa Xu <xjkxu@ucdavis.edu>)
Full Author List: Xu, Xiaosa[1 2]; Claeys, Hannes[2 3]; Chuck, George[4]; Thu, Tran[2]; Lang Vi, Son[2 5]; Demesa-Arevalo, Edgar[2 6]; Jackson, David[2]
[1] Department of Plant Biology, University of California, Davis, CA 95616, USA
[2] Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724, USA
[3] Current address: Inari, Industriepark 7A, 9052 Zwijnaarde, Belgium
[4] Plant Gene Expression Center, UC Berkeley, Albany, CA, 94710, USA
[5] Current address: Agricultural Genetics Institute, Hanoi, Vietnam
[6] Current address: Ingeniería Genética CINVESTAV Unidad Irapuato, Mexico

Plant development emerges from stem cell populations called meristems, which control organ initiation and branching. *RAMOSA3* (*RA3*), a classical maize developmental gene, controls inflorescence branching, and encodes a trehalose phosphate phosphatase enzyme. Our recent genetic and cell biology studies found that RA3 has a potentially non-enzymatic moonlighting function, since its phenotype can be uncoupled from catalytic activity. Furthermore, RA3 protein forms nuclear speckles, suggesting that it associates with transcriptional regulatory machinery in the nucleus. To tackle the mystery of the nuclear moonlighting function of RA3, we performed ethyl methyl sulfonate (EMS) mutagenesis and screening of *ra3* mutants, and identified an enhancer, *indeterminate spikelet1* (*ids1*). IDS1 is an AP2 type transcription factor that controls spikelet and floret development. By carefully examining the early developmental stages of *ra3;ids1* double mutants, we found that floral meristems were transformed into branches, rather than forming florets. We confirmed these findings by crossing *ra3* with additional *ids1* alleles and conducting allelism tests. Using *in-situ* hybridization, we found RA3 and IDS1 were co-expressed in the boundary regions between floral meristems, which was also supported by our single-cell transcriptomic profiling data. To further examine if IDS1 might be involved in the hypothetical transcriptional regulatory function of RA3, we checked for physical interactions and colocalization between RA3 and IDS1 *in planta*. Indeed, we found that RA3 and IDS1 proteins interact in nuclear speckles, reminiscent of the nuclear speckle localization of RA3. By further performing RNA-seq for *ra3* and *ids1* single and double mutants, we identified downstream candidate genes that were co-regulated by the putative RA3-IDS1 complex. Together, our data suggest that RA3 had a nuclear regulatory role in controlling inflorescence branching by interacting with the transcription factor, IDS1. Funding acknowledgement: National Science Foundation (NSF), UC Davis new faculty startup and Agricultural Experiment Station.

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## P77

## A spatiotemporal transcriptional map of maize lateral root formation

(submitted by Yaping Zhou <yzhou2@uni-bonn.de>)
Full Author List: Zhou, Yaping[1]; Hochholdinger, Frank[1]
[1] Institute of Crop Science and Resource Conservation, Crop Functional Genomics, University of Bonn, Bonn, Germany, 53113

Maize lateral root initiation and formation from a postembryonic stem cell niche is a multi-step process, which involves the spatiotemporal coordination of gene expression in pericycle cells. Conventional whole organ transcriptome analyses provide limited insights into transcriptional networks orchestrating root development as they do not account for the heterogeneity of cell types in time and space. To dissect the transcriptomic regulation of lateral root development at single-cell resolution, we profiled the two lateral root defective cell identity mutants *lateralrootless1* (*lrt1*) and *rootless with undetectable meristem1* (*rum1*), along with their wild types. On average, we recovered 8,286 cells per single cell experiment and detected in total 27,136 active genes across all surveyed genotypes, with a median of 1,276 active genes detected per cell. Moreover, we applied a uniform manifold approximation and a projection algorithm and assigned the identified cells into 19 clusters. To infer cell-type annotations, we employed a combined automated and manual approach and assigned each cell to one of eight cell types based on known marker genes. The expression profiles of cluster specific genes were consistent with the expression patterns in previously published maize root scRNA-seq datasets which supports the accuracy of the annotation. Functional genetic analyses of differentially expression genes will help to identify novel regulators of lateral root development in maize. Additionally, to add spatial information and identify novel root-type-specific regulators controlling lateral root formation, we employed laser capture microdissection coupled with RNA-seq to profile the transcriptomic reprogramming of pericycle cells in the primary root of *rum1* mutant and the three main root types of the wild type at three different developmental stages. Overall, our study will identity conserved and distinct players involved in the lateral root stem cell niche establishment of the three main root types of maize.

Gene / Gene Models described: *lrt1*, *rum1*; Zm00001d026691, Zm00001d043878
Funding acknowledgement: Deutsche Forschungsgemeinschaft

## P78

## Assessing factors underlying variation in maize pollen grain size: Genetics, development and environment.

(submitted by Harrison Bell <bellhar@oregonstate.edu>)
Full Author List: Bell, Harrison S T[1]; Fowler, John E[1]
[1] Oregon State University, Corvallis, OR, USA 97331

For maize seed production, pollen must successfully contribute to double fertilization of the female gametes. In Ipomoea and Rhododendron, pollen grain size has been demonstrated to positively correlate with pollen outcomes (siring ability and pollen tube growth rate, respectively), but there is scant evidence to address this definitively in cereals. We are interested in the genetic, developmental and environmental factors influencing pollen grain size in maize, and the potential implications of these on pollen fitness and fertility. To address these questions, we have adopted a dual approach, taking advantage of a newly-developed pipeline ('PollenVision') to generate morphological measurements of hundreds of individual pollen grains from fixed pollen samples. First, we collected pollen from over 550 maize lines from the Wisconsin Diversity (WiDiv) panel. Preliminary results from the first 65 WiDiv lines to be imaged and analyzed demonstrate that maize pollen grain size tends to be fairly consistent across the diversity panel, clustering around the expected pollen grain diameter (~100 µm). However, we have identified a minimum of four WiDiv lines which exhibit pollen sizes that deviate from this average by at least 2 standard deviations, two with small pollen, and two with large pollen. Second, to identify non-genetic factors, we collected pollen from three inbred, one hybrid, and three gametophytic mutant lines at repeated intervals, assessing whether variation in size is influenced by developmental stage (e.g., early shed vs. late shed), or environment. Preliminary analyses indicate that the average day-to-day pollen grain size from a single plant can vary as much as 28%. Analyses to determine whether any of the detected variation is influenced systematically by identifiable factors will be presented. Determining whether pollen grain size differs dramatically across shed period or field season will help inform how best to collect and analyze pollen from the WiDiv lines.

Funding acknowledgement: National Science Foundation (NSF)

## P79

# Asymmetric signaling and transcription factor interactions generate robust nonrandom patterning in maize leaf margins

(submitted by Richie Eve Ragas <rgr86@cornell.edu>)
Full Author List: Ragas, Richie Eve G[1]; Evans, Lukas J[1]; Wu, Hao[2]; Scanlon, Michael J[1]
[1] Plan Biology, School of Integrative Plant Sciences, Cornell University, Ithaca, New York, 14853
[2] College of Agriculture, Nanjing Agricultural University, Nanjing, China

Grass leaf margins exhibit decidedly non-random wrapper-tucker patterning, where the outer (wrapper) margin consistently surrounds the inner (tucker) margin in a serially repeatable robust pattern. Genetic analyses reveal that *WUSCHEL-LIKE HOMEOBOX3* (*WOX3*) gene paralogs implicated in leaf mediolateral outgrowth [i.e. *NARROW SHEATH 1* (*NS1*), *NARROW SHEATH 2* (*NS2*), and *WOX3a*] are essential for this pattern. Higher-order *wox3* mutants display random leaf margin patterning in a dosage-dependent manner. Molecular studies reveal that *WOX3a* transcripts accumulate preferentially in wrapper margins, whereas the boundary-marker gene *CUP-SHAPED COTYLEDON2* (*CUC2*) is highly expressed in tucker margins. The data suggest that asymmetric *WOX3a* accumulation promotes wrapper identity whereas *CUC2* promotes tucker. Additionally, the auxin-efflux gene *PINFORMED1* (*PIN1a*) exhibits tucker-biased transcript accumulation and adaxialized protein localization, with signals extending tipward in tucker margins, suggesting a role for PIN1-mediated auxin transport in establishing asymmetric marginal patterning. Moreover, genetic analyses reveal that *LIGULESS1* (*LG1*), required for development of the ligule and auricle in grass leaves, also interacts with *WOX3* paralogs during marginal patterning. Remarkably, whereas *ns1 ns2* double mutants fail to develop overlapping leaf margins, *ns1 ns2 lg1* triple mutants restore leaf margin development and may form fused margins that fail to delineate a wrapper or tucker. These findings suggest that previously undescribed interactions between transcription factors regulating leaf and ligule outgrowth and boundary formation contribute to the robust patterning of grass leaf margins.

Funding acknowledgement: National Science Foundation (NSF)

## P80

# Catalytic and non-catalytic TREHALOSE-6-PHOSPHATE SYNTHASES (TPSs) interact with RAMOSA3 to control maize development

(submitted by Thu Tran <tran@cshl.edu>)
Full Author List: Tran, Thu[1]; Claeys, Hannes[2]; Michalski, Kevin[1]; Boumpas, Panagiotis[3]; Williams, Z'Dhanne[4]; Sheppard, Samatha[1]; Vi, Son[5]; Chou, Tsung Han[1]; Furukawa, Hiro[1]; Jackson, David[1]
[1] Cold Spring Harbor Laboratory, 1 Bungtown Road, Cold Spring Harbor, NY11724, USA
[2] Inari, Industriepark 7A, 9052 Zwijnaarde, Belgium
[3] Heidelberg University, Centre for Organismal Studies (COS) Heidelberg, Department of Developmental Biology, Heidelberg, Germany
[4] Salivary Disorders Unit, National Institute of Dental and Craniofacial Research, National Institute of Health, Bethesda, MD, USA
[5] Faculty of Biotechnology, Chemistry and Environmental Engineering, PHENIKAA University, Yen Nghia, Ha Dong, Hanoi 12116, Vietnam

Trehalose-6-phosphate (T6P) is a key regulator of plant signaling networks, and coordinates growth by influencing carbon allocation, stress responses, architecture, and developmental transitions. T6P is the intermediate of trehalose biosynthesis mediated by T6P-synthases (TPSs) and T6P-phosphatases (TPPs). Plants harbor small families of *TPS* and *TPP* genes; while all TPPs are catalytic active, most plant TPSs are non-catalytic, suggesting they have regulatory functions. Here, we show that non-catalytic TPSs form a tripartite complex with catalytic TPSs and TPPs to control enzymatic activity and development.Maize mutant *ramosa3* (*ra3*) increases inflorescence branching and RA3 encodes a catalytic TPP. To investigate RA3 molecular mechanism, we screened for interactors, and found that it interacts with two non-catalytic TPS1, TPS12. *tps1* and *tps12* mutants enhance *ra3* phenotypes, suggesting their interaction is biologically significant. Interestingly, we found that TPS1 also interacts with the two maize catalytic TPS11 and TPS14. We knocked out these genes using CRISPR-Cas9, and the double mutants are embryo lethal. However, reducing active TPS expression in the *ra3; tps1; tps12* triple mutant background modifies its phenotype, supporting the idea interaction between three classes of proteins.To ask if TPS-TPP interactions affect enzyme activity, we performed a coupled enzyme assay, and found that the non-catalytic TPS1 stimulated the activity of RA3 and TPS14. This result suggests that RA3, TPS1, and TPS14 form a complex, and we confirmed after purifying the three proteins from insect cells. We used AlphaFold to predict that the TPS domains of TPS1 and TPS14 initiate this complex. To confirm this prediction, we co-expressed TPS1 and TPS14 and visualized heterotetramer complex formation by cryo-electron microscopy. From these results, we propose that the TPS1 TPS14 heterotetramer is important for both enzymatic activity and complex formation. These results provide insights into the structural basis and stoichiometry of TPS-TPP interactions, which have not been studied in any organism.In summary, we show a maize TPP (RA3) functions in a complex with both non-catalytic and catalytic active TPSs, and the non-catalytic TPS stimulates the activity of the active enzymes. Our research provides insights for the first time into the combined activity of the two major trehalose gene classes in plant development.

Funding acknowledgement: National Science Foundation (NSF)

## P81

### Cell-based high throughput screening in maize

(submitted by Kenneth Birnbaum <ken.birnbaum@nyu.edu>)
Full Author List: Birnbaum, Kenneth, D.[1]; Vissers, Graeme[1]; He, Jianjie[1]
[1] New York University, Center for Genomics and Systems Biology

We have been working on methods to perform whole genome screens on plant cells for functional discovery. These are akin to the Perturb-Seq and other related techniques that have been developed in metazoans. However, plants pose several specific challenges that need to be addressed. For one, a key aspect of cell-based whole genome screens is that perturbations—typically the delivery of CRISPR-components—are delivered in a pool of guide RNAs that target thousands of genes. Thus, it is essential that each cell take up only one or a very small number of perturbations. However, efficient transient transformation in plants requires high concentrations of DNA or delivery components that result in many components transfecting each cell. This makes screens hard or impossible to interpret. We have been working on this multiplicity of transfection problem and I will present our approaches to overcome this issue. In addition, I will present results on combinatorial screens and microfluidic systems to combine specific environmental and chemical conditions that provide specific conditions for cell division. These techniques have been used to induce specific cell identities in culture and perform combinatorial screens on the auxin signaling machinery. These sets of techniques offer a new opportunity to increase the throughput of screens to test for the genetic components that give rise to specific traits. I will discuss how cell-based screens can be designed to assess relevant traits.

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

## P82 @caldwed

### Cell-specific insights into fungicide mode of action and host resistance to improve tar spot management in maize

(submitted by Denise Caldwell <caldwed@purdue.edu>)
Full Author List: Caldwell, Denise L.[1]; Iyer-Pascuzzi, Anjali[1]
[1] Purdue University, Lafayette, IN, 47909

Tar Spot is a significant yield-limiting disease of maize (*Zea mays*) in the United States caused by the ascomycete *Phyllachora maydis*. Understanding the mechanisms of *P. maydis* colonization and evaluating control measures is critical for effective disease management. Growers currently use multiple commercially available fungicides to manage Tar Spot; however, the mechanisms and their efficacy at the microscopic level have yet to be examined. Thus, this set of studies aims to use histological and microscopic techniques to investigate the colonization process of *P. maydis* and assess the effectiveness of a commercially available, three-mode-of-action fungicide in susceptible and resistant maize hybrids. Three experiments were conducted to achieve these objectives. In the first study, we established a timeline for tissue collection from a susceptible maize genotype inoculated with *P. maydis*. Samples were collected at multiple time points across 18 days. In the second study, we evaluated the application timing of a commercially available, three-mode-of-action fungicide to inhibit *P. maydis* colonization and the development of reproductive structures in a susceptible hybrid. Lastly, we investigated colonization differences between susceptible and tolerant maize hybrids to identify morphological traits associated with reduced fungal spread and reproduction. This research aims to bridge a crucial knowledge gap by offering cell-specific insights into the mechanism of fungicidal activity and host resistance to *P. maydis*. Our findings will help inform fungicide application protocols and hybrid selection strategies for maize growers, ultimately contributing to improved disease management that will mitigate the economic impact of Tar Spot while promoting sustainable agricultural practices.

# P83

## Characterizing vasculature reconnection in maize "Twin-Grafts"

(submitted by David Zimmerman <zimmerman@cshl.edu>)
Full Author List: Zimmerman, David J.[1 2]; Heeney, Michelle[3]; Ferrieri, Richard A.[4 5 6 7]; Schueller, Michael J.[3 4]; Jackson, David[1]; Studer, Anthony J.[2]
[1] Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 11724
[2] Department of Crop Sciences, University of Illinois Urbana-Champaign, Urbana, IL 61801
[3] School of Integrative Plant Science, Cornell University, Ithaca, NY 14853
[4] Missouri Research Reactor Center, University of Missouri, Columbia, MO 65211
[5] Department of Chemistry, University of Missouri, Columbia, MO 65211
[6] Division of Plant Science and Technology, University of Missouri, Columbia, MO 65211
[7] Interdisciplinary Plant Group, University of Missouri, Columbia, MO 65211

Grafting is a technique that has been used in agriculture for millennia to combine two genetically distinct crop varieties to create a plant with beneficial shoot and root traits. Previously it was thought that graft compatibility was an exclusive trait of dicotyledonous species, however a recent study reported a method of grafting in monocots using imbibed seeds and kernels. We developed a protocol in maize and confirmed symplastic connection between primary root and shoot in both homo- and heterografts. However, this method requires destruction of the grafted shoot to confirm successful connection of the tissues. Here we present results from a novel shoot/shoot grafting method in which two individual maize shoots are fused at the mesocotyl during germination. Fused shoots were genotyped using molecular markers and MicroCT imaging was performed to confirm cell-cell connection. Phloem-xylem connectivity was also confirmed by administering radioactive $^{11}CO_2$ to a source leaf on one shoot and measuring phloem transport of [$^{11}$C]-photosynthate down the shoot to the roots and back up the second twin-shoot. Gamma ray counting and autoradiography were used for these measurements. In subsequent studies, we will investigate whether twin-grafting can rescue seminal and shoot-borne root development in *rootless concerning crown and seminal roots (rtcs)* maize mutants. We expect that with its ease of use and visual scoring coupled with the high rate of shoot/shoot fusion our method will become the standard grafting protocol for maize. Grafting in maize will allow researchers to unravel long distance signaling networks, secondary metabolite production, and source-sink dynamics throughout the maize lifecycle.

Funding acknowledgement: National Science Foundation (NSF), University of Missouri Research Council

# P84

## Chemical imaging reveals metabolic responses to salt-stress in maize roots

(submitted by Andrea Sama <asama@ucsd.edu>)
Full Author List: Sama, Andrea M[1]; Cahill, Sinead B[1]; Luo, Shihong[1]; Meng, Yifan[3]; Noll, Sarah E[3]; Zare, Richard N[3]; Shah, Pavak[2]; Dickinson, Alexandra Jazz[1]
[1] University of California, San Diego, La Jolla, California
[2] University of California, Los Angeles, Los Angeles, California
[3] Stanford University, Stanford, California

Metabolite signaling regulates many developmental and stress responses in plants, however, critical metabolite-driven links between stress and development remain an open area of exploration. To investigate metabolites involved in maize development, longitudinal sections of root meristems, which encompass the developmental transitions from stem cells to differentiated tissue, were imaged using mass spectrometry imaging (MSI) techniques. We used desorption electrospray ionization (DESI)-MSI and matrix-assisted laser desorption ionization (MALDI)-MSI, to generate high-resolution (20-50 µm) images of metabolites along the root developmental gradient. We developed a computational workflow to analyze our DESI-MSI data to identify compounds of interest and cluster metabolites based on their developmental enrichment patterns. This method enabled untargeted analysis of MSI data with consideration of developmental localization. We then applied this pipeline to characterize conserved enrichment patterns across maize varieties with different salt stress tolerances. Our approach led to the identification of low abundance compound localized to the meristem and elongation zone. Treatment with this compound enhances primary root growth under salt conditions in maize. Further characterization using Arabidopsis revealed that this treatment increases elongation zone length with treatment and has a potential effect on lateral root capacity. Visualizing the spatial localization of metabolites using MSI will continue to reveal candidate metabolites that bridge stress response and development. Characterizing the biological role of these metabolites, through a combination of chemical and genetic screens, will provide insights into the mechanisms that plants utilize to address stress and promote development enabling the generation of more resilient crop lines.

Funding acknowledgement: National Institutes of Health (NIH), Howard Hughes Medical Institute

# P85

## Comprehensive single-cell profiling of plant shoot stem cells uncovers key insights for functional studies and trait gene discovery

(submitted by Xiaosa Xu <xjkxu@ucdavis.edu>)
Full Author List: Xu, Xiaosa[1 2]; Passalacqua, Michael[2]; Rice, Brian[3]; Demesa-Arevalo, Edgar[2 8]; Kojima, Mikiko[4]; Takebayashi, Yumiko[4]; Sakakibara, Hitoshi[5]; Gallavotti, Andrea[6]; Gillis, Jesse[2 7]; Jackson, David[2]
[1] Department of Plant Biology, University of California, Davis, CA 95616, USA
[2] Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724, USA
[3] Department of Agronomy and Horticulture, University of Nebraska, Lincoln, NE, 68583, USA
[4] RIKEN Center for Sustainable Resource Science, Suehiro 1-7-22, Tsurumi, Yokohama 230-0045, JAPAN
[5] Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya 464-8601, Japan
[6] Waksman Institute of Microbiology, Rutgers University, Piscataway, NJ 08540, USA
[7] Department of Physiology, University of Toronto, Toronto, Ontario, M5S 3E1, CANADA
[8] Current address: Ingeniería Genética CINVESTAV Unidad Irapuato, Mexico

Stem cells in plant shoots are a rare population of cells responsible for producing leaves, fruits, and seeds, which are vital sources of food and bioethanol. Uncovering regulators expressed in these stem cells is crucial for informing crop engineering strategies to boost productivity. However, progress is limited by genetic redundancy and pleiotropy. Single-cell analysis has emerged as a powerful tool for identifying regulators expressed in specific groups of cells. Our recent studies leveraged single-cell analysis to identify distinct developmental domains in maize ear inflorescence. Using single-cell gene co-expression networks, we predicted genetic redundancy and discovered novel developmental markers highly associated with yield traits. Despite these advancements, accessing plant shoot stem cells remains challenging. Recent single-cell analyses of plant shoots have faced challenges in capturing these cells at a large scale or detecting stem cell regulators such as *CLAVATA3* (*CLV3*) and *WUSCHEL* (*WUS*). In this study, we finely dissected stem cell-enriched shoot tissues from maize and Arabidopsis for single-cell RNA-seq profiling. Optimized protocols enabled the efficient recovery of approximately 5,000 *CLV3*-expressing cells and 1,000 *WUS*-expressing cells, leading to the identification of hundreds of genes enriched in stem cells in both species. A single-cell cross-species analysis revealed conserved stem cell-enriched genes and cell types, which we validated using high-resolution multiplex spatial gene expression technology. We functionally characterized a family of conserved stem cell-enriched genes orthologous to the human *SERPINE1* mRNA-binding protein (SERBP1), a key ribosome-associated factor in humans. Additionally, we profiled maize stem cell overproliferation mutants and uncovered two families of sugar kinase genes as candidate targets of *WUS*. One family was found to function in cytokinin hormone homeostasis in stem cells. Our large-scale single-cell profiling serves as a valuable resource for mining stem cell regulators and understanding hidden regulatory mechanisms in plant shoot development. These stem cell regulators explained significant heritable variation and were enriched in genome-wide associations with maize yield component traits, providing insights to guide future crop engineering and improvement efforts. **Funding Acknowledgment:** NSF, UC Davis new faculty startup and Agricultural Experiment Station.

Gene / Gene Models described: *ZmSERBP1*, *ZmSERBP2*; GRMZM2G055276, GRMZM2G116282
Funding acknowledgement: National Science Foundation (NSF)

## P86

## Cross-species transcriptome comparison reveals candidate transcription factors for brace root development

(submitted by Taran Kermani <kermani@udel.edu>)
Full Author List: Kermani, Taran A.[1 2]; Sparks, Erin[1 3 4]
[1] Department of Plant and Soil Sciences, University of Delaware, Newark, DE 19713, USA
[2] Department of Biological Sciences, University of Delaware, Newark, DE 19713, USA
[3] Division of Plant Science and Technology, University of Missouri, Columbia, MO 65211, USA
[4] Donald Danforth Plant Science Center, St. Louis, MO 63132, USA

Brace roots are stem-borne nodal roots produced from above ground stem nodes in various grasses (Poaceae), including maize (Zea mays) and sorghum (Sorghum bicolor). These closely related species share many homologous genes and developmental gene expression patterns. However, few genes have been identified as regulators of brace root development in either species. To identify candidate regulators of brace root development, RNAseq and differential gene expression (DGE) analyses were performed on brace roots in three developmental stages (induction, initiation, and emergence) for both species. Transcription factors were selected as the focus of this analysis due to their well-known role as developmental regulators. Comparison of the maize and sorghum DGE analyses is expected to reveal homologs that are core regulators (regulate brace root development in both species) and species-specific regulators. ARFs (auxin response factors), WRKY, NAC, EREB (ethylene-responsive element binding), and various flowering-related transcription factors were identified from maize DGE analyses. Comparison of maize and sorghum homolog differential expression will reveal whether the identified maize transcription factors are core regulators of brace root development. The most highly differentially expressed core regulators will be prioritized for confirmation studies. Species-specific expression patterns may underlie morphological and functional differences between maize and sorghum brace roots, so additional confirmation studies will be performed for species-specific regulators. An understanding of brace root developmental regulation is important for the production of maize and sorghum lines with greater root lodging resistance and anchorage, and thus increasing crop yield to support the growing world population.

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

## P87

## Cytological, genomic, and comparative analysis of DNA replication in maize and sorghum

(submitted by Hank Bass <bass@bio.fsu.edu>)
Full Author List: Bass, Hank W.[1]; Wheeler, Emily[2]; Akram, H. Sara[1]; Wear, Emily E.[2]; Mickelson-Young, Leigh[2]; Lezama, Christine L.[1]; Concia, Lorenzo[3]; Thompson, William F.[2]; Hanley-Bowdoin, Linda K.[2]
[1] Department of Biological Science, Florida State University, Tallahassee, FL, USA
[2] Plant and Microbial Biology, North Carolina State University, Raleigh, NC, USA
[3] Texas Advanced Computing Center, University of Texas, Austin, TX, USA

DNA replication in plants and other eukaryotes is a highly organized process in which different genomic regions are duplicated in a defined order. Replication time (RT) is an inherent property of individual genome segments, rather than a simple function of their linear order on a chromosome. This process has been studied much less in plants than in animals. We developed a system using the root tips of maize seedlings that allows us to study replication timing with both cytological and molecular detail. This work is supported by the NSF PGRP program and involves collaboration among NCSU, FSU, and TACC. Our findings indicate that the replication process in maize significantly differs from that in animals. Notably, we do not observe the megabase-scale RT domains commonly observed in mammalian systems. Instead, we find a distinct spatial distribution of replication activity within the nucleus, where early- and mid-S replicating regions are closely interspersed throughout most of the nucleoplasm. Our cytological and molecular analyses (including Hi-C) reveal that early- and mid-replicating chromatin in maize define two different euchromatin compartments, contrasting with results from animal systems. Ongoing work aims to define initiation regions in maize, building on our earlier research in Arabidopsis, which showed significant differences from animal systems. Additionally, we are comparing RT programs in developmentally different meristems and between various maize cultivars and sorghum. For this purpose, we have characterized a simpler system for obtaining RT profiles. Ultimately, we hope to use this system to score progeny of crosses between parental cultivars differing in their RT profiles, and thus to begin a genetic analysis of RT determinants in maize.
Funding acknowledgement: National Science Foundation (NSF)

## P88

## Designing form and function into research devices

(submitted by Doris Cao <dcao@udel.edu>)
Full Author List: Cao, Doris[1 2]; Sparks, Erin[2]
[1] Newark Charter School, Newark, DE 19711
[2] University of Delaware, Newark, DE 19716

Research devices are valuable tools for measuring the biomechanics of maize. These devices allow researchers to quantify root and stalk systems within maize to detect plant diseases and assess effects of different plant genotypes. The Sorghum and Maize Under Rotational Force (SMURF) device is designed to measure root system stiffness by extending a lead screw to cause a 6 degree rotation at the base of a plant. The Plant Pusher device is designed to push the plant to measure stalk mechanics and stiffness.
Several modifications were implemented on these devices to enhance the functionality and efficiency for more comprehensive data collection. For the SMURF device, changes were made to prevent inconsistent robotic movements and increase data resolution. Additionally, the device's flexibility and versatility were improved with the implementation of a user input field in the interface that allows for custom distance movement. These modifications led to more thorough tests and minimized the damage inflicted on the plant. In the future, these implementations will enable the SMURF to be scaled down to perform tests on younger plants for earlier diagnostics. The Plant Pusher device's interface was redesigned to reduce user-error and improve the usability in the testing environment. This included improved color schemes, rearranged buttons, and a reduction in the number of buttons by combining functions. These changes made the interface more accessible and adaptable to different environments. In the future, this interface will be further scaled down to make it more universally accessible through an Android app. As a result of these improvements, testing times were reduced and device usability increased.

Funding acknowledgement: National Science Foundation (NSF)

## P89

## Determining flowering phenotypes and expression signatures of temperate and tropical maize grown in short and long day field environments

(submitted by Fernanda Ghenov <fghenov@hawaii.edu>)
Full Author List: Ghenov, Fernanda[1]; Hinrichsen, Jacob T.[2]; Schoenbaum, Gregory R.[2]; Sanchez, Itzel E.[1]; Yu, Jianming[2]; Muszynski, Michael[1]
[1] Department of Tropical Plant and Soil Sciences, University of Hawai'i at Mānoa, Honolulu, HI, USA
[2] Department of Agronomy, Iowa State University, Ames, IA, USA

The critical transition in the maize life cycle involves shifting from vegetative growth, characterized by leaf production from the shoot apical meristem (SAM), to reproductive growth, where the SAM produces floral organs. Known as the floral transition, this stage occurs early in development and is influenced by internal cues and external signals such as photoperiod and temperature. The timing of the floral transition significantly impacts flowering, a crucial adaptive trait subject to selection. Maize varieties adapted to temperate latitudes exhibit photoperiod insensitivity, flowering consistently under both short (SD) and long (LD) day conditions. In contrast, tropical maize, retaining much of the photoperiod sensitivity of its progenitor teosinte, requires SD to flower within a similar timeframe as temperate maize. To comprehensively understand the photoperiod sensitivity of tropical maize and its effects on various developmental traits, we conducted field-based phenotyping of specific temperate and tropical maize genotypes for three consecutive years under LD and SD. This poster illustrates how the growing environment influences SAM reprogramming, total leaf count, and days to flowering. Our analysis explores the interplay between photoperiod and temperature on flowering timing. We also present preliminary gene expression data, showing diurnal patterns before and after the floral transition under SD conditions.

Funding acknowledgement: National Science Foundation (NSF)

## P90

### Differential expression of nitrogen transporters contributes to uptake differences between aerial and soil brace roots

(submitted by Emilia Pierce <emiliap@udel.edu>)
Full Author List: Pierce, Emilia[1]; Sparks, Erin[1 2 3]
[1] Department of Plant and Soil Sciences, University of Delaware, Newark, DE 19713, USA
[2] Division of Plant Science and Technology, University of Missouri, Columbia, MO 65201, USA
[3] Donald Danforth Plant Science Center, St. Louis, MO 63132, USA

Brace roots are above ground stem-borne roots that play an important role in anchorage and nutrient uptake. While their role in anchorage is well defined, the nutrient uptake capacity of brace roots has not been well studied. To quantify the nitrogen uptake rate of brace roots that penetrate soil (soil brace roots) and brace roots that do not penetrate soil (aerial brace roots), nitrogen uptake assays were performed using three different types of labelled $^{15}N$. Data collected from these assays show that aerial brace roots are able to take up more nitrogen than soil brace roots in both field- and greenhouse-grown maize, and in field-grown sorghum. We hypothesized that this unexpected outcome is caused by differential expression of nitrogen transporters in aerial versus soil brace roots. To test this hypothesis, RNA-sequencing was performed on B73 aerial and soil brace roots. Twenty-four nitrogen transporters were found to be significantly differentially expressed between the two root types, twelve of which were higher in aerial brace roots. These results were compared to a publicly available root-type RNA-sequencing dataset, and several nitrogen transporters were found to be differentially expressed in both datasets. Additionally, ten nitrogen transporters were analyzed by RT-qPCR in brace root samples of five different genotypes. Four of these transporters were significantly differentially expressed between aerial and soil brace roots across genotypes. These findings support our hypothesis that differential expression of nitrogen transporters may contribute to a variation in nitrogen uptake between aerial and soil brace roots.

Funding acknowledgement: National Science Foundation (NSF), Royal Society

## P91

### Effects of phenylboronic acid on maize root development: Boron-related but rhamnogalacturonan II-independent?

(submitted by Liuyang Chu <lchu@uni-bonn.de>)
Full Author List: Chu, Liuyang[1]; Matthes, Michaela[1]
[1] INRES-Crop Functional Genomics, University of Bonn, Friedrich-Ebert-Allee 144, Bonn, NRW, Germany, 53113

Boron is an essential micronutrient for plant development and its deficiency negatively affects actively growing tissues. While boron's role in maize shoot development is well studied, its impact on maize root development is less understood. Boron predominantly resides in primary cell walls and it dimerizes two rhamnogalacturonan II (RG-II) monomers to form borate-crosslinked RG-II dimers. RG-II is a subunit of pectin and RG-II crosslinking is the only well-characterized function of boron. Experimentally inducing boron deficiency remains challenging due to boron´s ubiquity. Phenylboronic acid (PBA), a boric acid analog, has been suggested as a tool to mimic boron deficiency by occupying boron-binding sites and interference with RG-II dimerization.
To evaluate whether PBA can mimic boron deficiency-induced phenotypes in maize, time-course experiments were performed. Both PBA and boron-free treatments reduced lateral root density and only slightly affected primary root length, with PBA inducing stronger defects. In vitro, however, PBA did not affect boric acid-mediated RG-II dimerization, indicating that PBA-induced defects are independent of RG-II crosslinking. Notably, 3,3-diaminobenzidine staining results indicated increased $H_2O_2$ production in PBA-treated maize roots compared to the non-PBA controls. Since PBA can be oxidized by $H_2O_2$ to phenol and boric acid, we further assessed the influence of H2O2 and phenol on the observed PBA-induced root phenotypes. Both compounds did not reduce lateral root density. Additionally, seedling assays with oxidation-resistant or non-aromatic boronic acids induced similar root defects as PBA. These observations implied that the boric acid group is responsible for the observed phenotypes and suggested additional targets of boron beyond RG-II. Our study showed that PBA induces phenotypes similar to boron deficiency in maize roots, emphasizing a tissue-specific role of boron during maize root development. PBA, however, did not affect RG-II crosslinking and therefore will allow the identification of novel RG-II-independent functions of boron in the future.

Funding acknowledgement: German Research Foundation (DFG)

## P92
## Exploring small molecule regulation of root development in *Zea Mays*

(submitted by Abigail Tripka <atripka@ucsd.edu>)
Full Author List: Tripka, Abigail[1]; Lou, Shihong[1]; Meng, Yifan[2]; Zare, Richard N.[2]; Noll, Sarah E.[2]; Dickinson, Alexandra Jazz[1]
[1] University of California San Diego; San Diego, California, 92093
[2] Stanford University; Stanford, California, 94305

Advancing our knowledge of organismal development involves decoding the roles of small molecules in stem cell patterning. My lab applies chemical imaging to the maize root meristem to investigate fundamental principles that govern small molecule control of stem cell decisions, competency, and fate. Our Desorption Electrospray Ionization Mass Spectrometry Imaging (DESI-MSI) studies have found that visualization of small molecule localization can be used to predict novel biological regulators. I perform HPLC-MS/MS on maize root meristem extracts to elucidate the structures of unidentified meristem-enriched molecules. Through this approach, I have identified molecular signatures consistently localized to the maize root meristem across multiple varieties of maize, including a series of benzoxazinoids (BXs). BXs are abundant in globally important food crops like maize and wheat and are predominantly studied for their role in biochemical defense against various biotic stresses. However, the strong enrichment of BXs to the maize root meristem raises an interesting question: do BXs play a role in maize root development? Preliminary results indicate that a BX compound can promote maize seminal root growth, suggesting that BXs may play a role beyond defense.
Utilizing *Arabidopsis*, a plant that does not natively have a BX pathway, enables us to investigate BX functions further. My findings show that BXs can influence root system architecture in *Arabidopsis*, suggesting they may have functions in development. I am continuing to investigate the role of BXs in root development through phenotypic, transcriptomic, and proteomic analysis. Results of this study will further elucidate if BX genes have a role in development and are regulators of root meristem cell decisions. Uncovering small molecule regulators of development in both model organisms and crops has the potential to offer valuable contributions to our understanding of plant chemical biology and agriculture.

Funding acknowledgement: San Diego Match Fellowship, Quantitative Integrative Biology Fellowship

## P93 @julian_somers3
## Exploring the timing of the sporophyte-to-gametophyte transition (SGT)

(submitted by Julian Somers <jms53460@uga.edu>)
Full Author List: Somers, Julian[1]; Nelms, Brad[1]
[1] University of Georgia, Athens, GA, 30602

Plants alternate between multicellular haploid gametophyte and diploid sporophyte generations. The sporophyte-to-gametophyte transition (SGT) is a transition from sporophytic to gametophytic expression that occurs during gametophyte development. The SGT is defined by global changes in steady state transcript levels and involves various changes in genome regulation. In maize pollen, widespread gametophyte genome activation occurs between late unicellular microspore and bicellular microspore stages, setting the timing of the SGT around pollen mitosis I (Nelms & Walbot, 2022). The SGT sets the stage for active transcription of the haploid pollen genome, causing pollen to express phenotypes and undergo haploid selection. We are using single-cell genomics to explore the timing of the SGT, which controls the timing and scope of haploid selection. While we know the SGT occurs around pollen mitosis I in maize, we do not know if this timing is conserved in other species. To address this, we are determining the timing of the SGT in Arabidopsis, tomato, tobacco, and rice. This research will fill fundamental knowledge gaps in the current understanding of the haploid generation of plants, providing novel information relevant to breeding practices and agriculture.

Funding acknowledgement: National Institutes of Health (NIH)

## P94

## Genes that regulate azimuthal canopy re-orientation exhibit sensitivity to plant density

(submitted by Yan Zhou <yzhou86@iastate.edu>)
Full Author List: Zhou, Yan[1 2]; Hansen, Eric[1 2]; McCargar, Desere' L[2]; Hagedorn, Kara[2]; Jubery, Talukder Z.[3]; Ganapathysubramanian, Baskar[3]; Schnable, Patrick S.[1 2]
[1] Department of Agronomy, Iowa State University, Ames, Iowa 50011, USA
[2] Plant Science Institute, Iowa State University, Ames, Iowa 50011, USA
[3] Department of Mechanical Engineering, Iowa State University, Ames, Iowa 50011, USA

Canopy architecture contributes to the efficiency of solar radiation interception by crops. A significant feature of canopy architecture is the distribution of leaves around the stem of a plant relative to the axis of planting as viewed from above, i.e., azimuthal canopy orientation. Some genotypes exhibit the ability to alter their azimuthal canopy orientations during development. Genes associated with this re-orientation trait have been identified via GWAS; many of these genes are involved in light responses (see poster by Zhou et al.). Mutants of these genes (N=12) and wild-type controls were grown at two planting densities: 34,000 plants/acre (an agronomic density) and 8,000 plants/acre. UAV-acquired images of these plots were collected 60 days after planting (i.e., at approximately the time of tasseling). Azimuthal canopy orientations were then extracted from these images. As expected, when grown at the agronomic planting density, wildtype controls exhibited azimuthal canopy re-orientation towards the open interrow spaces. In contrast, at this planting density, such responses were reduced or even reversed in the mutants. In contrast, at 8,000 plants/acre, neither wild-type controls nor mutants exhibited azimuthal canopy re-orientation, presumably as a consequence of reduced shading. Interestingly, under the agronomic planting density in some of the mutants, crown root architectures were also altered relative to wild-type. These results indicate that genes involved in above-ground light responses can also affect root architecture.

Funding acknowledgement: National Science Foundation (NSF)

# P95

## Genetic control of lateral root branching in response to moisture

(submitted by Johannes Scharwies <joscha@stanford.edu>)
Full Author List: Scharwies, Johannes D[1]; Clarke, Taylor[1]; Zheng, Zihao[3]; Dinneny, Andrea[1]; Birkeland, Siri[4]; Veltman, Margaretha A[5]; Sturrock, Craig J[6]; Banda, Jason[6]; Torres-Martínez, Héctor H[1]; Viana, Willian G[1]; Khare, Ria[7]; Kieber, Joseph[7]; Pandey, Bipin K[6]; Bennett, Malcolm[6]; Schnable, Patrick S[3]; Dinneny, José R[1 2]
[1] Stanford University; Stanford, CA, USA 94305
[2] Howard Hughes Medical Institute; Stanford, CA, USA 94305
[3] Iowa State University; Ames, IA, USA 50011-1085
[4] Norwegian University of Life Sciences; Ås, Norway 1432
[5] University of Oslo; Oslo, Norway 0318
[6] University of Nottingham; Sutton Bonington, UK LE12 5RD
[7] University of North Carolina; Chapel Hill, NC, USA 27599

Plant development is highly influenced by signals from the environment. Below ground, complex root systems develop in response to these signals to facilitate efficient water and nutrient uptake. Understanding how signals from the environment shape root systems and how this led to the evolution of specific root system architectures will provide knowledge and tools to optimize plants for future climates. Our research focused on understanding how moisture distribution around roots controls the patterning of lateral root branches, which affects the overall shape of root systems. In a panel of 250 maize inbred lines, we characterized hydropatterning, the behavior in which lateral roots are preferentially initiated from root surfaces in direct contact with moisture and are suppressed on air-exposed surfaces. We observed that inbred lines from the non-stiff stalk subpopulation showed a higher propensity of initiating lateral roots from air-exposed surfaces compared to inbred lines from the tropical/subtropical subpopulation. Evolutionary analyses suggested that this difference between subpopulations was best explained by divergent selection. We used Genome and Transcriptome Wide Association Studies to identify candidate genes in maize that are associated with the hydropatterning behavior. Our analysis confirmed that auxin distribution and perception play an important role in hydropatterning. Additionally, we discovered genetic variation linked to *ZmFLA4* (*Zm00001eb367960*) which affects hydropatterning and leads to differences in the production of the gaseous plant hormone ethylene. Using mutants of orthologous genes in Arabidopsis, we confirmed that ethylene suppresses the formation of lateral roots on air-exposed root surfaces. Our findings provide the first comprehensive overview of phenotypic diversity for hydropatterning in a crop species and explore potential effects of selection through breeding. Moreover, we provide important evidence that ethylene plays a pivotal role in controlling lateral root formation in response to moisture.

Gene / Gene Models described: *FLA4*; Zm00001eb367960
Funding acknowledgement: Department of Energy (DOE), HHMI

## P96

## Genetic networks of maize phyllotaxy mutants

(submitted by Lander Geadelmann <landerg@iastate.edu>)
Full Author List: Geadelmann, Lander[1]; Unger-Wallace, Erica[1]; Strable, Josh[1]; Vollbrecht, Erik[1]
[1] Department of Genetics, Development, and Cell Biology; Iowa State University, Ames, Iowa

Regular patterned leaf initiation at the shoot apical meristem (SAM), or phyllotaxy, is a robustly buffered phenotype in plants, including maize. Few mutants have been identified in controlling maize's alternating pattern of leaf initiation. The recessive mutant aberrant phyllotaxy1 (abph1), a cytokinin signaling gene, can alter phyllotaxy wherein leaves are initiated on opposite sides of the plant in pairs offset by 90˚. This paired leaf phenotype is metastable however, and lost upon introgression into corn belt inbred lines, which is a bottleneck to the study of phyllotaxis in maize. We have identified transcription factor (TF) genes ereb130, ereb184 and paralogs whose mutants result in a more stable altered phyllotaxy. These TF mutant phenotypes have relatively high penetrance in standard inbred lines in which abph1 mutants are nearly normal. Two double mutants, ereb130;ereb184 and ereb130;abph1, both have increased penetrance and expressivity of disrupted phyllotaxy and phenocopy each other, suggesting together these three genes act in a phyllotaxy-related genetic pathway. Using Lexogen 3'Quantseq, RNA expression profiles for shoot apices of ereb130;ereb184, ereb130;abph1, their respective single mutants and nonmutant siblings, were obtained for differential expression analysis and Weighted Gene Correlation Network Analysis (WGCNA). Using these computational tools, modules of genes associated with altered phyllotaxy were discovered among these mutant genotypes. To further filter this -omics data, we employed Causal-WGCNA (CWGCNA) which uses a mediation-model to test which genes might be driving altered phyllotaxy versus which genes change expression as a downstream effect of altered phyllotaxy. We hypothesize that such "driver" candidate genes may be directly tied to a larger phyllotaxy-related genetic network. Mutant analysis of candidate genes is in progress.

Gene / Gene Models described: *ereb130*, *ereb184*, *abph1*; Zm00001eb005740, Zm00001eb058850, Zm00001eb076960
Funding acknowledgement: National Science Foundation (NSF), Iowa State University, ISU Crop Bioengineering Center

## P97

## Harnessing pluripotency: Identifying key regulators of plant cell regeneration

(submitted by Nicholas Francis <francini@oregonstate.edu>)
Full Author List: Francis, Nicholas[1]; Leiboff, Samuel[1]
[1] Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR, 97331, USA

Every cell contains the genetic blueprint necessary to form a complete multicellular organism, yet only pluripotent cells possess the ability to generate all cell types. In *Arabidopsis*, mesophyll protoplasts can dedifferentiate into proliferating tissue (callus), and ultimately regenerate into whole plants after hormone treatment. Maize protoplasts, however, are recalcitrant to regeneration, and callus regeneration is restricted to natural or induced embryonic cell types. Black Mexican Sweet (BMS) maize mesocotyl suspension cells are adapted to continuous growth as callus and remarkably have been documented to induce pluripotency and allow regeneration when co-cultured with protoplasts from other genotypes, a process mediated by unknown secreted factors. Preliminary morphological examination of BMS suspension cell cultures with fluorescent stains show that cells within BMS microcalli are morphologically diverse, produce distinct secondary cell wall decorations, and differentially accumulate lignin, suberin, and other cell wall components. We are using time-course imaging of protoplasts to investigate morphological changes associated with BMS co-culture. We tracked nuclear-localized YFP to analyze nuclear remodeling and cell division during BMS co-culture. Preliminary results suggest that BMS co-culture can rapidly impact nuclear organization, leading to multinucleate protoplasts within 16 hours of incubation. Ongoing work aims to connect these observations to cell identity and re-entry into the cell cycle. Future work will use machine learning models to identify latent morphological patterns, cluster cellular morphologies, and detect remodeling events. With a morphological understanding of maize protoplast and BMS co-culture, future work will use RNA sequencing at key time points to correlate gene expression profiles with observed cellular changes. BMS medium will be analyzed using metabolomic and proteomic techniques to identify secreted factors and candidate compounds tested for their ability to induce regeneration. Together, these efforts will clarify the transcriptional landscape and molecular signals regulating maize protoplast regeneration, offering new avenues for advancing crop genetic transformation.

Funding acknowledgement: National Science Foundation (NSF)

## P98

## Heat stress at tricellular stage inhibits maize pollen dehiscence

(submitted by Kevin Begcy <kbegcy.padilla@ufl.edu>)
Full Author List: Rana, Divya[1]; Kim, Taehoon[1]; Begcy, Kevin[1]
[1] Environmental Horticulture Department, University of Florida, PO Box 110670, Gainesville, FL 32611, USA

Global warming has caused a significant increase in the frequency and intensity of heatwaves and hot days worldwide. Exposure to high temperatures during the plant reproductive phase leads to dramatic decrease in seed production. Moreover, male reproductive organs including anthers and pollen are more sensitive to temperature fluctuations than female gametophytes. While the impact of heat stress at the tetrad, unicellular and bicellular stage of pollen development has been elucidated, it is not known how heat stress (HS) impacts the tricellular stage of pollen development. Using three maize (*Zea mays*) inbred lines (B73, W22 and A188), we imposed a moderate heat stress (35°C/25°C light/dark period) for 48 h on maize plants, specifically at the tricellular stage. We found that HS at the tricellular stage resulted in reduced starch content, decreased enzymatic activity, and reduced *in vitro* and *in vivo* pollen germination rates. In addition, HS delayed the anther dehiscence and significantly reduced the seed set of all three inbred lines. Transverse sectioning of heat stressed anthers demonstrate that heat stress delays the disintegration of endothecium layer, while non-stressed plants complete disintegration of the endothecium layer in anthers and thus leading to reduced pollen release. To understand the molecular basis of these phenotypes, we performed RNA-Seq analysis on anthers and pollen grains under HS and control conditions. Among the differentially expressed genes, HS yielded MADS2. This transcription factor is required for anther and pollen maturation in maize and accumulates in apoptotic bodies during anther dehiscence. Using transgenic plants carrying the ZmMADS2-green fluorescent protein (GFP) fusion protein under control of the *ZmMADS2* promoter, we performed Co-immunoprecipitation and ChIP-Seq assays to identify interactors and downstream genes of this gene.

Gene / Gene Models described: *MADS2*; Zm00001eb338060
Funding acknowledgement: United States Department of Agriculture (USDA)

## P99

### Influence of the temperature, light and nutritional status in infection by *Agrobacterium tumefaciens* in maize leaf transformation

(submitted by Maria Helena Faustinoni Bruno <mfaustinonibruno@gmail.com>)
Full Author List: Bruno, Maria Helena F[1 2]; Pinto, Maísa S[1]; Rios, Letícia[1]; Pauwels, Laurens[4]; Yassitepe, Juliana[1 3]; Arruda, Paulo[1 2]
[1] Genomics for Climate Change Research Center (GCCRC), Universidade Estadual de Campinas, Campinas, SP, Brazil,13083-875
[2] Departamento de Genética, Evolução, Microbiologia, e Imunologia- Instituto de Biologia, Universidade Estadual de Campinas, Campinas, Brazil, 13083- 970
[3] Embrapa Agricultura Digital, Campinas, SP, Brazil, 13083-886
[4] Department of Biotechnology, Faculty of Bioscience Engineering, Ghent University, Proeftuinstraat 86, Ghent, Belgium, 9000

Maize genetic transformation is an important method for developing new varieties. Alternative explants, like mature embryo slices and leaf-base tissue segments, have been explored in maize to overcome the labor-intensive embryo transformation protocol. Environmental conditions significantly impact plant development, and the explant quality is essential for successful transformation. We investigated how temperature, light, and nutritional status influence *Agrobacterium tumefaciens* infection in maize leaves used for transformation. We used a leaf-based protocol, where B104 maize plants were cultivated in 16h/light- 8h/dark photoperiod, testing intensities light of 80, 160, and 240 µmol light, and temperature of 21°C, 24°C and 27°C. Plants were categorized as healthy and unhealthy based on visual symptoms (size and nutritional deficiency), and the leaves were used for transformation. We used *Agrobacterium* strain EHA105 recA$^-$ equipped with the ternary vector pVS1-VIR2 and a binary vector expressing the β-glucuronidase (GUS) reporter gene to monitor transient transformation. Infection area by *Agrobacterium tumefaciens* were quantified using ImageJ software, and nutritional analysis was performed through atomic absorption spectrometry. Image analysis showed that healthy plants exhibited higher infection rates than unhealthy plants, and the better condition was healthy plants in 160 µmol light and 24°C, where the infection rate covered 5,07% of the total area. In contrast, plants grown under 240 µmol light showed the least favorable conditions with a low infection between 0,02-0,6% of the total area. Nutritional analysis demonstrated that higher infection rates are associated with healthy plants. Pearson Correlation highlighted a positive relation between infection area and calcium (0,52), manganese (0,55), and iron (0,44). Additionally, this analysis revealed a negative relation between infection area and light (-0,30), suggesting that high light intensity may reduce the success of *Agrobacterium* infection. These results indicate that health, environmental conditions, and nutritional status play a significant role in the success of infection and maize transformation.

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## P100

### Investigating TANGLED1-mediated division site maintenance across land plant lineages

(submitted by Makayla Drew <makaylad@ucr.edu>)
Full Author List: Drew, Makayla[1]; Rasmussen, Carolyn[1]
[1] University of California, Riverside. Riverside, CA. United States, 92507

Establishment of the proper division plane, or where the new cell wall will be placed upon completion of cytokinesis, is essential for proper growth and patterning in land plants1. In plants, this division plane is positively marked by a number of division site proteins, including the microtubule-binding protein TANGLED1(TAN1). Previous analyses of TAN1 function in the tan1 air9 mutant background, a synthetic division plane mutant, have demonstrated that TAN1 alone can rescue all mutant phenotypes observed in the synthetic double mutant. Disruption of TAN1 interaction motifs for interaction with PHRAGMOPLAST-ORIENTING-KINESIN1 (POK1) abolishes TAN1-mediated rescue of phragmoplast angle variance phenotypes seen in the double mutant. This project aims to shed light on the evolution of the TAN1-POK1 interaction across land plants, and hypothesize how important this interaction has been in phragmoplast guidance of other land plants.

Funding acknowledgement: National Science Foundation (NSF)

## P101 @PairwisePL

### Leveraging LRR receptor gene editing for enhanced ear morphology in maize

(submitted by Rob Meister <rmeister@pairwise.com>)
Full Author List: Meister, Rob[1]; O'Connor, Devin[2]; Crawford, Brian[1]; Graham, Nathaniel[1]; Guffy, Sharon[1]; Mathew, Lolita[1]; Mojica, Julius[1]; Ochsenfeld, Cherie[1]; Pham, Gina[1]; Watts, Joseph[1]; Bartlett, Madelaine[3]; Karlson, Dale[4]; Slewinski, Tom[5]; Lawit, Shai[1]
[1] Pairwise; Durham, NC, USA 27701
[2] Colorado State University; Fort Collins, CO, USA 80523
[3] University of Massachusetts; Amherst, MA, USA 01003
[4] Terrana Biosciences; Raleigh, NC, USA 27615
[5] Bayer Crop Science; Chesterfield, MO, USA 63017

Increases in harvestable yield in row crops are essential to meet growing food demands and support sustainable agronomic practices. One promising avenue for boosting maize productivity is through improvements of ear morphology traits, such as kernel row number. Published literature indicates that some Leucine Rich Repeat (LRR)-containing receptors play key roles in regulating meristematic activity in inflorescence meristems. We employed the Pairwise Fulcrum™ Gene Editing platform to make targeted edits within critical kinase functional domains and protein complex residues of selected LRR genes to modify or disrupt the signaling capacity that drives meristematic proliferation. In collaboration with Bayer Crop Science, we assessed the phenotypic outcomes of these edits under various growth conditions, monitoring kernel row number and other ear-related traits. Our analyses revealed observable effects on kernel row number across multiple edited lines. Notably, certain edits conferred dominant effects, enabling trait enhancement without requiring homozygosity. These dominant gene edits are particularly advantageous for breeding pipelines, as they accelerate the introgression of beneficial alleles while reducing the time and resources needed to generate stable lines. Overall, our results demonstrate gene editing of LRR-containing receptors can influence inflorescence meristem activity and thereby improve ear morphology traits in maize. This work underscores the potential of precision genome editing to drive advances in crop productivity and supports the broader goals of sustainable agronomic practices.

## P102

### Localized changes in developmental phase-transition on a recurrent morphogenetic regulatory network enables maize leaf angle variation

(submitted by Ruqiang Zhang <rz444@cornell.edu>)
Full Author List: Zhang, Ruqiang[1]; Char, Si Nian[2]; Liu, Hua[2]; Evans, Lukas, J.[1]; Tang, Lie[3]; Li, Xianran[4]; Lubkowitz, Mark[5]; Yang, Bing[2]; Yu, Jianming[6]; Scanlon, Michael, J.[1]
[1] Plant Biology Section, School of Integrative Plant Science, Cornell University, Ithaca, NY, USA 14853
[2] Division of Plant Sciences, University of Missouri, Columbia, MO, USA 65201
[3] Department of Agricultural and Biosystems Engineering, Ames, IA, USA 50011
[4] USDA-ARS, Wheat Health, Genetics, and Quality Research Unit, Pullman, WA, USA 99164
[5] Saint Michael's College, Colchester, VT, USA 05439
[6] Department of Agronomy, Iowa State University, Ames, IA, USA 50011

Maize leaves are serially produced from shoot meristems, and each leaf comprises a proximal sheath surrounding the stem and a distal blade projecting from the stem. The blade/sheath boundary is demarcated by an epidermal fringe of ligule tissue and a multi-layered auricle, which forms a triangular-hinge that generates leaf-angle. Leaf-angle describes the bending of the leaf blade away from the stem, a key architectural trait and a target for crop improvement. Maize inbred lines show distinct variation in leaf angle within the canopy. Specifically, upper-canopy leaves exhibit increased leaf angle in inbred EP1, but leaf angle is decreased (more upright) in upper-canopy leaves of inbred B73. In contrast, inbred B104 displays equivalent leaf angle in the upper and lower canopies. Although candidate genes regulating maize leaf angle have been identified, little is known about the control of leaf angle variation across the canopy. Transcriptomic landscapes of emerging ligule/auricles from the lower and upper canopy were generated for these three inbred lines, and dynamic modeling of a recurrent, morphogenetic gene regulatory network was used to probe the mechanism of leaf angle variation in maize. We introduce a model describing “phase-change speed” to explain how the rates of phase transition can be stage-specifically modulated during the recurrent emergence of the auricle. In the model, auricle development is affected by the speed of vegetative phase change, where faster rates of transition from juvenile to adult phases leads to reduced auricle development and small leaf angle, and slower rates of phase change enlarge the auricle resulting in increased leaf angle. The model is supported by genetic analyses of leaf angle in maize mutants that disturb the rate of developmental phase change.
Funding acknowledgement: National Science Foundation PGRP

## P103
## MALE STERILE CONVERTED ANTHER 1 (MSCA1), Hypoxia and TGA Transcription Factors

(submitted by Faith Kanana Mbiti <faith.kanana@ur.de>)
Full Author List: Mbiti, Faith Kanana[1]; van der Linde, Karina[1]
[1] Cell Biology and Plant Biochemistry, University of Regensburg, 93053 Regensburg, Bavaria, Germany.

Male germline formation in maize (*Zea mays*) is a complex and highly regulated process that is influenced by genetic and environmental factors. MALE STERILE CONVERTED ANTHER 1 (MSCA1), is involved in the differentiation of meristematic layer 2 cells into archesporial cells. In *msca1* mutant anthers, archesporial cells and their surrounding somatic cell layers fail to establish, instead vasculature like tissue is formed resulting in male sterility. TGA transcription factors, are known for their involvement in plant defense and stress responses and have emerged as potential regulators of reproductive development in the recent past. MSCA1 has been shown to interact with FEA4, a TGA transcription factor and together they regulate meristem size. In *A. thaliana*, ROXY1/2, the orthologs of MSCA1 interact with TGA9, TGA10, and PAN (member of the TGA transcription factor family) in the process of anther and petal formation. There are 19 TGA transcription factors encoded in the maize genome, but so far, no known *tga* knockout mutant resembling the *msca1* anther phenotype has been reported. Therefore, we aim to address this question by exploring the interaction between MSCA1 and TGA transcription factors, and their combined role in male germline development in maize. RT-qPCRs and RNA *in situ* hybridization assays revealed expression of *TGA10*, *TGA11*, *LG2*, *TGA2*, and *TGA17* in anthers during archesporial cell fate specification. Using Yeast Two-Hybrid assays and a Fluorescence Resonance Energy Transfer (FRET) approach, we identified direct physical interactions between MSCA1 and 8 TGA transcription factors. Currently, maize loss of function mutants and reporter lines of *TGA*10, *TGA11*, *TGA2*, and *TGA17* are being generated. These maize lines will offer more insights into the molecular mechanism governing male fertility in maize.

Funding acknowledgement: DAAD (German Academic Exchange Service)

## P104 @KamilinyPlants
## Making good connections: How transverse veins span the maize leaf vascular network

(submitted by Maria Camila Medina Montes <medinamm@oregonstate.edu>)
Full Author List: Medina, Maria Camila[1]; Chuck, George[2]; Leiboff, Samuel[1]
[1] Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331, USA.
[2] Department of Plant and Microbial Biology, UC Berkeley, Berkeley, CA 94720, USA.

Maize leaves develop several classes of parallel vein including midvein, laterals, intermediates, and small veins, as well as one class of perpendicular vein, the transverse veins. We are using transverse veins as a model to uncover mechanisms of vascular development across developmental time and spatial domains within the leaf. Using classical anatomical tools, we observed that mature transverse veins consist of xylem, phloem, and bundle sheath and seamlessly span all parallel vein classes, although they initiate after parallel veins. After developing an anatomical/morphological staging system that separates plastochron, 'P' stages into up to 3 substages, we used the *ZmPIN1a-YFP* auxin transport marker line to determine that transverse veins initiate during P6.1 at the tip of the pre-blade and progress through the pre-sheath by P10. We observed that transverse veins begin with paired transverse anticlinal divisions along parallel vein procambia, producing two cytoplasmically dense "initials" with prominent nuclei and increased *ZmPIN1a-YFP* expression. Transverse anticlinal divisions and expression of *ZmPIN1a-YFP* eventually completes a vascular trace, in a process which can span multiple cell files. We observed expression of the *DR5::erRFP* auxin signaling maker line during late stages of transverse vein differentiation, suggesting that initiation may not require transcriptional auxin signaling. Although the sheath lacks small veins, transverse vein recruitment appears to follow a conserved program in pre-blade and pre-sheath. As the pre-sheath expands, transverse veins transition from an orderly grid-like pattern to a more organic, ladder-like shape. We observed growth of the pre-auricle domain in P10, after transverse vein patterning is complete in pre-blade and pre-sheath, suggesting that the auricle is devoid of transverse veins because it develops after a required temporal-developmental signal. Using our staging system as a guide, we performed RNA-Seq on 62 libraries representing 416 leaf primordia from SAM-P3 to P8 to identify transcripts which predict transverse vein initiation.

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

## P105
**[Removed at the request of the author]**

## P106
### New tools for visualizing phasiRNA biogenesis in maize anthers via hybridization chain reaction, immunofluorescence, and multiplex super-resolution microscopy.

(submitted by Allyson Angermeier <aangermeier@danforthcenter.org>)
Full Author List: Angermeier, Allyson[1]; Czymmek, Kirk J[1]
[1] Donald Danforth Plant Science Center; St. Louis, Missouri, 63132

Phased small interfering RNAs (phasiRNAs) are 21- or 24-nucleotide (nt) small RNAs with major developmental impacts but poorly defined roles. Previous studies revealed that loss of phasiRNAs in maize anthers causes male sterility, highlighting their crucial role in anther development. In maize anthers, 21-nt phasiRNA biogenesis initiates when Argonaute 5c (AGO5c) interacts with a "trigger" miRNA2118 to identify phasiRNA precursor transcripts, which are then cleaved in a phased manner by Dicer-like 4 (DCL4). These 21-nt phasiRNAs are initially produced in the anther epidermis but translocate to all cell types. To gain insights into phasiRNA biogenesis and functions in maize anthers, we optimized sample preparation, molecular localization, and imaging tools to enhance our understanding of this important pathway. We employed hybridization chain reaction (HCR), a form of fluorescence *in situ* hybridization with greater specificity and substantial signal amplification, to study the localization of target specific RNA species at the single-molecule level. We used a multiplexing approach (several rounds of labeling) in combination with HCR, immunofluorescence, and lattice structured illumination microscopy (SIM) to localize the different molecular classes of the phasiRNA pathway during anther development. We assessed the localization of AGO5c, DCL4, and RNA-dependent RNA polymerase 6 (RDR6) mRNAs, phasiRNA precursors, and miRNA2118 along with new antibodies for AGO5a, AGO1a, AGO1e, DCL4 and RDR6. Our study demonstrated that HCR, combined with immunofluorescence and super-resolution lattice SIM imaging, was a robust method for dissecting the phasiRNA biogenesis pathway components cellular and tissue distributions with remarkable specificity. Our methods can be generally applied to the cellular analysis of various RNA species and protein localization. We thank Blake C. Meyers and Virginia Walbot for scientific guidance, and Joanna Porankiewicz-Asplund and Agrisera, Part of Olink Group, for providing antibodies. This work was funded by NIH GM151302.

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## P107 @NelmsLab

### Pizza theory! Meristem organization restricts the spread of new mutations among the offspring

(submitted by Brad Nelms <nelms@uga.edu>)
Full Author List: Scherer, Justin[1]; Park, Sungjin[1]; Gaisie, Ahema[1]; Nelms, Brad[1]
[1] University of Georgia; Athens, GA 30606

Mutations accumulate throughout development, contributing to cancer, aging, and evolution. After a mutation occurs, its consequences depend heavily on how widely it can spread through the cell population. For instance, a mutation in a meristematic stem cell can exert a much wider effect on the organism and potentially be transmitted to the offspring. We are using the maize Mutator (Mu) transposon as a model of new mutation. By adapting Mu sequencing assays for molecular counting, it is possible to track the spread of *de novo* Mu insertions across tissues of the same plant or between generations. Here, we present evidence that the radial symmetry of plant embryonic development and subsequent meristem growth maintains multiple distinct cell lineages throughout the plant's life. This avoids population bottlenecks in the critical stem cell niche and thus reduces how often offspring inherit the same new (untested) mutation, a form of evolutionary bet-hedging. Finally, we find that a similar strategy – an effort to avoid population bottlenecks in the germline – can also explain quirks of animal development, from *C. elegans* to humans. We propose the 'distributive germline' as a general developmental mechanism to preserve genetic diversity in the offspring and constrain the spread of new mutations.

Funding acknowledgement: National Institutes of Health (NIH)

## P108

### Positional cell divisions, growth, and recruitment contribute to cell-fate acquisition at a developmental boundary in the maize leaf

(submitted by Lukas Evans <Le95@cornell.edu>)
Full Author List: Evans, Lukas J.[1]; Palos, Kyle R.[2]; Fitzgerald, Elizabeth A.[1]; Zhang, Ruqiang[1]; Schmitz, Robert J.[3]; Sylvester, Anne W.[4]; Nelson, Andrew D.L.[1 2]; Scanlon, Michael J.[1]
[1] School of Integrative Plant Science, Cornell University, Ithaca, NY USA 14853
[2] Boyce Thompson Institute, Ithaca, NY USA 14853
[3] Department of Genetics, University of Georgia, Athens, GA USA 30602
[4] Marine Biological Laboratory, Woods Hole, MA USA 02543

Developmental boundaries are essential features of complex metazoans, although boundary formation is incompletely understood. The blade-sheath boundary of maize leaves comprises two distinct tissues, an epidermally-derived ligule and a hinge-like auricle, which demarcate the upward-projecting blade from the clasping, proximal sheath. Previous morphological and transcriptomic analyses focused on ligule initiation; mechanisms of later-staged ligule development remain unclear, while there are few prior studies of auricle ontogeny. Here, we combine dynamic imaging and single-cell transcriptomics to examine the mechanisms of ligule outgrowth and auricle patterning at this leaf boundary. Unlike the rapid and frequent epidermal cell divisions accompanying initiation and outgrowth of ligule, the auricle comprises all cell layers and initiates as a thin, rectangular band of small isotropic cells with comparatively few mitotic figures. Notably, the auricle doesn't assume its characteristic triangular shape, shortest near the midrib and widest at the leaf margins, until relatively late in development and correlates with increased frequency of cell division toward the margins. Moreover, vascular anastomosis occurs at the distal edge and the auricle lacks stomata. Single-cell RNA-seq data identifies markers denoting sheath, auricle, ligule, and blade identity, enabling pseudotime projections of cell differentiation at the ligule/auricle leaf boundary. Candidate transcription factors were utilized in DNA Affinity Purification Sequencing; predicted binding sites are supported by single-cell transcriptomics. Lineage analysis show that the auricle is clonally-related to the blade. Our data supports a model wherein cells in the proximal, primordial blade are recruited by differential-gene-expression to undergo patterned cell divisions and growth to form an auricle.

Funding acknowledgement: National Science Foundation (NSF)

## P109 @vishadinie

### Response of autophagy-deficient maize *atg10* mutants to heat stress

(submitted by Vishadinie Jayasinghe <jmvisha@iastate.edu>)
Full Author List: Jayasinghe, Vishadinie[1]; Tang, Jie[1]; Agbemafle, William[2]; Bassham, Diane C[1]
[1] Department of Genetics, Development and Cell Biology, Iowa State University, Ames, IA, USA, 50011
[2] Roy J. Carver Department of Biochemistry, Biophysics and Molecular Biology, Iowa State University, Ames, IA, USA, 50011

Autophagy, a highly conserved cellular process that degrades and recycles damaged proteins and organelles, plays a crucial role in maintaining cellular homeostasis under stress conditions. In maize (*Zea mays*), heat stress is a significant environmental challenge that adversely affects growth, development, and yield. Autophagy is involved in mitigating the damaging effects of heat stress by promoting the removal of misfolded or denatured proteins, damaged organelles, and toxic byproducts that accumulate under elevated temperatures. While evidence from other plant species suggests that autophagy contributes to thermotolerance, the relationship between autophagy and heat stress in maize remains poorly understood. We have investigated the role of autophagy in heat stress responses in maize by examining autophagy-deficient *atg10* mutants. We observed that transcripts encoding heat shock proteins (HSPs) are upregulated in *atg10* mutants under heat stress under field conditions. This upregulation of *HSP* genes in the absence of functional autophagy indicates a potential compensatory response in the *atg10* mutants, possibly reflecting an altered heat stress adaptation mechanism. To further elucidate the molecular mechanisms, we performed RNA sequencing (RNA-seq) and identified differentially expressed genes (DEGs) in wild-type (WT) and *atg10* mutant plants under heat stress. In summary, this study illuminates the current understanding of the role of autophagy in heat stress tolerance in maize, highlighting the mechanistic insights and the potential for autophagy-based strategies in improving heat stress resilience in maize.

Gene / Gene Models described: *atg10*; Zm00001eb425740
Funding acknowledgement: National Science Foundation (NSF), Walter E and Helen Parke Loomis Fund

## P110 @thanduanlung

### SBP mutants have an expanded competence zone for brace root initiation

(submitted by Thanduanlung Kamei <thanduan@udel.edu>)
Full Author List: Kamei, Thanduanlung[1]; Sparks, Erin[1 2 3]
[1] Department of Plant and Soil Sciences, University of Delaware, Newark, DE., USA
[2] Division of Plant Science and Technology, University of Missouri, Columbia, MO, USA
[3] Donald Danforth Plant Science Center, St. Louis, MO USA

Brace roots develop from the stem pulvinus in above-ground nodes, but the molecular pathways regulating stages of brace root development remain poorly explored. RNA sequencing of young stem nodes at three stages of brace root development identified SQUAMOSA Promoter Binding Protein (SBP) genes (e.g., unbranched2 (UB2), unbranched3 (UB3), and tasselsheath4 (TSH4)) as negative regulators of brace root development. These transcription factors exhibit high expression levels in nodes without brace root primordia and expression is subsequently reduced at nodes with primordia present and primordia emerging. Loss-of-function sbp mutants show an expanded competence zone (i.e., wider stem pulvinus) that results in a second, albeit incomplete, whorl of brace roots developing at each node. Double and triple sbp mutants have a more complete second whorl of brace roots, suggesting an additive effect of SBP genes to restrict the competence zone. However, the expanded competence zone is lost in Corngrass1 (Cg1), which overexpresses the miRNA targeting these SBP genes and could be considered a complete loss of SBP function. To elucidate the molecular mechanisms underlying the expanded competence zone, we dissected nodes into two regions, S1 (basal whorl) and S2 (expanded whorl), from W22, tsh4, and ub2/ub3 plants, and performed RNA sequencing. Our analysis revealed that the transcript profiles of S1 and S2 regions in the wild type were highly divergent, whereas the single tsh4 and double ub2/ub3 S1 and S2 profiles were increasingly similar. This dataset can be leveraged to identify the genes responsible for brace root induction within the competence zone. The differentially expressed genes from this analysis are being interrogated to define the regulatory networks controlling brace root development.

Gene / Gene Models described: *ub2, ub3, tsh4*; Zm00001d031451, Zm00001d052890, Zm00001d020941
Funding acknowledgement: National Science Foundation (NSF)

## P111

## STR2 – a plant half-sized ABCG transporter with a multifaceted role in AM symbiosis

(submitted by Liam German <ltg27@cam.ac.uk>)
Full Author List: German, Liam[1]; Manley, Bethan[1]; Sgroi, Mara[1]; Jaquet, Jennifer[2]; Li, Bailin[2]; Paszkowski, Uta[1]
[1] Crop Science Centre, Department of Plant Sciences, University of Cambridge, Cambridge, CB3 0LE, United Kingdom.
[2] Corteva Agriscience, 7300 NW 62nd Ave, Johnston, IA 50131, USA

Investigation of a *Zea mays* mutant generated in a forward genetic screening led us to discover an 'early' symbiosis phenotype, with the colonisation of arbuscular mycorrhizal (AM) fungi blocked at an early stage. The mutant was named *independent of AM symbiosis* (*ina*). The colonisation of *ina* plants by *Rhizophagus irregularis* was further tested through a nurse plant system and supplementation of wild-type root exudates. Meanwhile, positional cloning and CRISPR/Cas9 gene editing was used to identify the which gene causes the AM symbiosis phenotype. Growth with both wild-type nurse plants or root exudates partially recovered the colonisation of *ina* plants, but surprisingly the individual plants that were colonised in these conditions only developed stunted arbuscules. Positional cloning and CRISPR/Cas9 gene editing revealed the gene responsible was *STUNTED ARBUSCULE 2* (*STR2*) – encoding a half-sized ABCG transporter. Heterodimers of STR2 and STR1 are believed to mediate lipid delivery to the fungus at the periarbuscular membrane, with the stunted arbuscule phenotype already reported in Medicago and Rice. However, this new early colonisation phenotype identified in *ina* indicates STR2 has a second function before symbiosis starts, perhaps through interaction with another ABCG partner. Considering this, here we also present results from our efforts to identify potential binding partners of STR2 that act during the early colonisation role. Since colonisation of *ina* can be complemented with exudates, this suggests that an unknown molecular component (potentially lipidic) is needed for AM fungus to initiate symbiosis. This work re-contextualizes our understanding of the STR2 protein's role.

Gene / Gene Models described: *STR2, STR1*; Zm00001d043722, Zm00001d024075
Funding acknowledgement: BBRSC

## P112

## Sex and death: Mapping and characterization of several novel *tassel seed* mutants with altered sex determination

(submitted by Brian Zebosi <zebosib@oregonstate.edu>)
Full Author List: Zebosi, Brian[1]; Lunde, China[3]; de Neve, Amber[2]; Bonney Arku, Comfort[2]; Bartlett, Madelaine[2 4]; Leiboff, Samuel[1]
[1] Department of Botany and Plant pathology, Oregon State University
[2] University of Massachusetts, Amherst
[3] University of California, Berkeley
[4] The Sainsbury Laboratory, Cambridge University

Maize produces unisexual flowers selective abortion of carpel and stamen primordia in the tassel and ear. Sex determination defective mutants have shown that a complex network of transcriptional factors, microRNAs, phytohormones, and environmental cues control this process. Interestingly, carpels are aborted in both the tassel and in the lower floret of the ear. To identify novel carpel suppression genes and understand the link between these processes across inflorescence types, we are characterizing nearly 40 novel maize *tassel seed* (*ts*) sex determination mutants from the Maize Genetics Cooperative Stock Center. By pairing bulk segregant analysis (BSA) and whole genome sequencing, we have developed a robust pipeline for F2 mapping even in backgrounds where the parent of origin is unknown through comparisons with multiple NAM founder genomes. Our preliminary analysis has identified mapping intervals for 10 of these mutants, 4 are likely novel alleles of *ts1*, 5 are likely novel alleles of *ts2*, and 1 maps to a novel interval. We also mapped the dominant *Ts3* mutant to the long arm of a 14 Mbp interval on chromosome 1 with approximately 365 genes, which we hypothesize contains structural variation. Using a sensitized *grassy tillers1* (*gt1*) background with a mild failed carpel abortion phenotype, we used EMS mutagenesis followed by BSA sequencing to identify synergistic regulators of carpel suppression, termed *rapunzel** (*rzl*-1* and *rzl*-2*). We localized these mutations to short arm of chromosome 6. Further genetic and bioinformatic analyses are ongoing to verify these mutants and understand the underlying mechanisms regulating sex determination and carpel suppression in maize.

Gene / Gene Models described: *ts1, ts2*; Zm00001eb081610 ,Zm00001eb013890
Funding acknowledgement: National Science Foundation (NSF), United States Department of Agriculture (USDA)

# P113

## Spatial analysis of stomatal patterning can quantify component traits tied to developmental genetics of stomatal density in maize

(submitted by John Hodge <jgerardhodge@gmail.com>)
Full Author List: Hodge, John G[1 2]; Leakey, Andrew DB[1 2 3 4]
[1] Carl R. Woese, Institute of Genomic Biology, 1206 W. Gregory Dr., University of Illinois Urbana-Champaign, Urbana, IL 61801, USA
[2] Center for Advanced Bioenergy and Bioproducts Innovation, 1206 W. Gregory Dr., University of Illinois Urbana-Champaign, Urbana, IL 61801, USA
[3] Department of Plant Biology, University of Illinois Urbana-Champaign, Urbana, IL 61801, USA
[4] Center for Digital Agriculture, University of Illinois Urbana-Champaign, Urbana, IL 61801, USA

Stomata regulate leaf gas exchange of CO2 and water vapor, i.e. water use efficiency (WUE). Stomatal development has been studied as a model system for understanding regulation of cell fate, and to revealing how stomatal patterning influences leaf gas exchange. The genetic basis of stomatal patterning is mainly understood from composite traits like stomatal density (SD) and stomatal index (SI). Stomatal patterning is flexible, so multiple genotypes can possess equivalent SD or SI while possessing different epidermal cell sizes/patterns. Therefore, analyzing the component traits underlying SD would be valuable. Optical tomography and AI-enabled computer vision can now rapidly generate large data sets of the relative positions of stomata on the epidermis. This study used such data from a B73 x Ms71 mapping population to develop a novel method that statistically summarizes the self-repeating Stomatal Patterning Phenotype (SPP). A set of key SPP component traits describing variation along the lateral and longitudinal axes of the leaf were studied both using structural modeling and genetic mapping to assess trait interactions. Thirteen quantitative trait loci (QTL) for SD also associated with SPP-derived patterning traits with two of the major QTL segregating into distinct longitudinal effects that govern the stomatal file system (Chr 9) whereas lateral effects affect the spacing of these files along the width of the leaf (Chr 1). Overall, these spatial methods provide a case study for leveraging AI automated annotations of high-throughput phenotyping data to atomize histology into orthogonal components, which can be identified by their distinct genetic drivers, and help improve our understanding of complex composite traits, like stomatal density, through their component precursors.

Funding acknowledgement: Department of Energy (DOE)

## P114

## Supporting the next generation: bHLH transcription factor complexes regulating maize anther development

(submitted by Lily O'Connor <loconnor@danforthcenter.org>)
Full Author List: O'Connor, Lily[1]; Angermeier, Allyson[1]; Teng, Chong[2]; Czymmek, Kirk[1]; Walbot, Virginia[3]; Meyers, Blake[2]
[1] Donald Danforth Plant Science Center; Saint Louis, MO, 63132
[2] University of California Davis; Department of Plant Sciences; Davis, CA 95616
[3] Stanford University; Department of Biology; Stanford, CA, 94305

Anthers are the male reproductive organ of flowering plants, and they produce the male gametophyte, pollen. Fully differentiated pre-meiotic maize anthers are composed of four concentric somatic cell layers that surround the central meiocytes. The tapetal cell layer is the innermost somatic cell layer and the tapetum provides crucial support to the meiocytes through pollen maturation. To support meiocyte development, the tapetum undergoes significant physiological changes including becoming binucleated, secretory, and finally undergoing programed cell death. A cascade of four ACT-like domain containing basic helix-loop-helix (bHLH) transcription factors (TF), Male Sterile (MS) 23, MS32, bHLH122, and bHLH51 regulate tapetal cell differentiation and development. Null allele mutations in any one of these bHLH TFs results in complete male sterility due to a failure of the tapetal cell layer to fully differentiate. To test the direct role of bHLH122 in regulating tapetal cell development, we generated a line containing an epitope tagged copy of bHLH122. Using this line, we show that bHLH122 is expressed and localizes to tapetal cell nuclei during two stages of anther development, an early meiotic stage and a post-meiotic stage. Co-immunoprecipitation of bHLH122 followed by mass spectrometry shows that during each stage of expression, bHLH122 forms distinct protein-protein interactions. bHLH122 co-precipitates primarily with MS23 and MS32 during the early meiotic stage and bHLH51 during the post-meiotic stage. The stage specific interactions of bHLH122 govern the promoter elements bHLH122 complexes bind, thereby defining the transcriptional regulatory effect of bHLH122 across anther development.

Gene / Gene Models described: *Ms23*, *Ms32*, *bHLH122*, *bHLH51*; Zm00001eb332170, Zm00001eb106620, Zm00001eb251650, Zm00001eb208200
Funding acknowledgement: National Science Foundation (NSF)

## P115

## TARGET OF RAPAMYCIN regulates gene expression across developmental gradients

(submitted by Michael Busche <busche.michael@gmail.com>)
Full Author List: Busche, Michael[1]; Martinez, Ryan E[1]; Menon, Sannidhi[1]; Brunkard, Jacob O[1]
[1] University of Wisconsin, Madison; Madison, WI USA 53703

The rate of development in maize is stable under many stresses but its tempo changes significantly when nutrients, including nitrogen or carbohydrates, are limiting. TARGET OF RAPAMYCIN (TOR) is a deeply conserved eukaryotic protein kinase that monitors cellular resources to coordinate growth and development with the availability of these essential nutrients. To explore the impact of nutrient availability on maize development, we designed a nutrient-limiting assay to precisely toggle nutrient-sensitive developmental responses in growing maize embryos. We performed functional genomic experiments including transcriptomics, quantitative proteomics and phosphoproteomics, metabolomics, polysome profiling, and Ribo-seq on embryos grown under these conditions. Here, we report the changes that occur in the translational landscape during maize development in response to changes in nutrient availability. Ribo-seq revealed dramatic changes in the translational efficiency of many important developmental genes while polysome profiling showed dramatic shifts in translation globally in response to changes in TOR activity. To complement these experiments and further study the impact of changing developmental stage on TOR activity, we divided developing maize leaves into equal sections from base to tip, creating a developmental and physiological gradient. We repeated functional genomic experiments described above in this system and found that TOR activity and TOR-dependent gene expression is heavily influenced by developmental stage.

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

# P116
## TERMINAL EAR1 (TE1) couples maize development with stress responses

(submitted by Nandhakumar Shanmugaraj <shanmug@cshl.edu>)
Full Author List: Shanmugaraj, Nandhakumar[1]; Tran, Thu[1]; Iohannes, Sessen D[1]; Chen, Richelle[5]; Wu, Peipei[1]; Claeys, Hannes[3]; Arevalo, Edgar D[4]; Kitagawa, Munenori[2]; Liu, Lei[2]; Jackson, David[1]
[1] Cold Spring Harbor Laboratory, 1 Bungtown Road, Cold Spring Harbor, NY, USA
[2] Huazhong Agricultural University, Wuhan, China
[3] Inari, Industriepark 7A, Ghent, Belgium
[4] CINVESTAV-IPN, Unidad Irapuato: Irapuato, Guanajuato, Mexico
[5] Cardozo School of Law, NY, USA

TERMINAL EAR 1 (TE1) is a maize RNA-binding protein that controls various aspects of plant development, including leaf initiation, phyllotaxy, plant height, and inflorescence architecture. RNA immunoprecipitation sequencing (RIP-Seq) with TE1-Yellow Fluorescent Protein (YFP)-expressing maize ear primordia revealed that TE1 binds mRNAs of key maize developmental regulators such as *TASSELSHEATH4*, *UNBRANCHED2*, and *UNBRANCHED3*, which function in shoot branching and organ patterning. Interestingly, no significant changes were found in the mRNA levels of most TE1 targets in *te1* mutants. In contrast, shotgun proteomics and western blot analyses revealed that the corresponding protein levels of TE1-bound mRNAs were elevated in *te1* mutants, suggesting that TE1 might post-transcriptionally regulate its targets by repressing translation. We also identified TE1-YFP interacting proteins using proteomics, and Gene Ontology analysis of these interactors revealed enrichment in stress granule assembly, regulation of translation and mRNA stabilization categories. Consistent with this, subcellular localization studies revealed that functional TE1-YFP fusion proteins localize in cytoplasmic puncta and co-localize with stress granule markers under heat shock. Time-course imaging of TE1-YFP ear primordia found that these puncta rapidly enlarged following heat stress, further implicating TE1's involvement in stress response mechanisms.To determine if TE1 functions in stress responses, we tested germination following heat treatments, and found that *te1* mutants were more sensitive to heat stress. Prolonged heat stress of *te1* seedlings also found differential responses and significantly earlier flowering than the wild-type plants. Our results suggest that TE1 binds to developmental mRNAs and sequesters them in cytoplasmic granules as part of a mechanism to negatively regulate plant development and mediate the tradeoff between plant growth and stress responses. Further mechanistic understanding of maize TE1 could provide new insights into the interplay between development and stress adaptation and suggests that manipulating TE1 function could enhance crop resilience.

Gene / Gene Models described: *te1*; Zm00001eb144140
Funding acknowledgement: National Science Foundation (NSF)

## P117

### The Wisconsin Crop Innovation Center: a public resource for crop transformation and gene editing research

(submitted by Dayane Cristina Lima <dclima@wisc.edu>)
Full Author List: Lima, Dayane Cristina[1]; Kaeppler, Heidi[1 2]; Mahoy, Jill[1]; Lor, Vai[1]; Collier, Ray[1]; Walter, Nathalie[1]; Hahn, Emily[1]; McGinty, Alyson[1]; Matheka, Jonathan[1]; Carlson, Alvar[1]; Kaeppler, Shawn[1 2]
[1] Wisconsin Crop Innovation Center, University of Wisconsin-Madison, 8520 University Green, Middleton, WI 53562
[2] Department of Plant and Agroecosystem Sciences, University of Wisconsin-Madison, 1575 Linden Drive, Madison, WI 53706

The Wisconsin Crop Innovation Center (WCIC) at the University of Wisconsin-Madison is the largest public sector, fee-for-service plant transformation center in North America. Committed to best stewardship practices and quality management, the center has earned the Excellence Through Stewardship (ETS) certification in plant biotechnology. WCIC offers advanced genetic transformation and gene-editing services to both academic and private sector clients. The center specializes in a wide range of crops, including maize, soybean, and other key agricultural species, supporting cutting-edge research and innovation in plant biotechnology. A project begins with contract negotiations between the University of Wisconsin and the client's institution. Following contract execution, the transformation pipeline may be initiated. Typically, the process starts with the design and assembly of plasmid vectors. Once a transformation vector is created or delivered, Agrobacterium stocks are prepared for use in generating transgenic plants. Regenerated plantlets from culture are then transferred to the greenhouse for analysis using the selection marker. For overexpression vectors, copy number analysis services are available to identify single-locus events. The plants are advanced, self-pollinated or outcrossed, and $T_1$ seed is generated. WCIC also offers $T_1$ seed advancement and screening to produce homozygous $T_1$ plants, which can be used to bulk $T_2$ seed for direct trials or for the creation of hemizygous transgenic hybrid $T_2$ seed through outcrossing with a second inbred parent. For editing vectors, we offer both shipping of $T_0$ leaf tissue samples and are developing internal edit detection capacity to increase the likely delivery of edited events. We also offer a seed-expressed color gene marker to assist segregation of the edited trait from the transgenic editing locus in the $T_1$ seed. All transgenic seed will be shipped to the client following the successful completion of the required USDA-APHIS permits for interstate seed shipment. Details can be found at cropinnovation.cals.wisc.edu.

## P118

### The cis-regulatory evolution of GRASSY TILLERS1 (GT1) in the grass family

(submitted by Hailong Yang <hailongyang@umass.edu>)
Full Author List: Yang, Hailong[1]; Gallagher, Joseph P[1 2]; Lally, Kyra[1]; McKnight, Ronan[1]; Amundson, Kirk[1]; De Neve, Amber[1]; Facette, Michelle[1]; Bartlett, Madelaine[1 3]
[1] Department of Biology, University of Massachusetts Amherst, Amherst, Massachusetts, USA 01003
[2] Forage Seed and Cereal Research Unit, US Department of Agriculture, Agricultural Research Service, Corvallis, OR 97331
[3] Sainsbury Laboratory, Cambridge University, Cambridge, United Kingdom

Cis-regulatory evolution is critical for divergence in gene function. Evolutionarily conserved non-coding sequences (CNSs) likely mark key cis-regulatory regions, yet how CNSs modulate gene function remains largely unknown. Here we examine CNSs upstream of GRASSY TILLERS1 (GT1), which encodes a Class I HD-ZIP transcription factor, and has conserved roles in multiple grasses including Hordeum vulgare (barley), Brachypodium distachyon (brachypodium), Zea mays (maize), and Triticum aestivum (wheat). GT1 is a pleiotropic domestication gene that represses growth in multiple developmental contexts, including tillers, floral organs, and ear buds. Prolificacy1.1 (prol1.1) is a major quantitative trait locus (QTL) upstream of GT1 that regulates ear number. Our evolutionary tracing of CNSs suggests that the distal region of prol1.1 contains ancient CNSs shared by most grasses (41 - 52 Mya), while the proximal region contains CNSs that arose more recently (27 - 42 Mya). To determine the function of these CNSs, we made CRISPR/Cas9 alleles in prol1.1. Our preliminary data shows that the proximal region of prol1.1 accounts for more variation in ear number than the distal region. Ongoing work is dissecting the molecular basis of this functional difference. We are also editing CNSs in the homologous prol1.1 region of brachypodium to determine how these CNSs may modulate phenotypic variation in the grasses. Our work will reveal how cis-regulatory evolution modulates gene expression.

Gene / Gene Models described: *GT1*; Zm00001eb007950
Funding acknowledgement: National Science Foundation (NSF), United States Department of Agriculture (USDA)

## P119

## The *enhancer of spi1* (*eos1*) gene functions in maize tassel development by regulating auxin transport

(submitted by Prameela Awale <pa96f@umsystem.edu>)
Full Author List: Awale, Prameela[1]; Ensor, Maya[1]; Robil, Janlo[1]; McSteen, Paula[1]
[1] University of Missouri-Columbia, MO, 65211

In maize, auxin regulates the initiation of all axillary meristems (branch, spikelet pair, spikelet, and floral meristem) in the tassel. Mutations in genes involved in auxin synthesis, transport or signaling cause defects in the initiation of all types of axillary meristems in the maize inflorescence. *enhancer of spi1* (*eos1*) mutants have fewer tassel branches compared to wildtype and a barren patch of missing spikelets at the base of the main spike. We identified two mutator induced *eos1* mutant alleles and verified that insertions in the coding region of the gene cause the tassel phenotype. Scanning electron microscopy of immature tassels show regions of missing, aborted and single spikelets, which indicates initiation defects, likely related to auxin. Confocal microscopy using the auxin transport reporter *pZmPIN1a*::*ZmPIN1a*:*YFP* revealed that PIN localization is absent in the missing axillary meristems in immature mutant tassels indicating that *eos1* may be involved in auxin transport. To dissect the role of *eos1* in regulating auxin we generated double mutants of *eos1* with the auxin biosynthesis mutant *sparse inflorescence 1 (spi1)* and the auxin transport mutant *barren inflorescence 2 (bif2)*. The *spi1* mutant makes few tassel branches and defective spikelets in the tassel, while the *bif2* mutant make fewer branches and spikelets than *spi1*. The *eos1*; *spi1* double mutants showed a synergistic interaction with severe tassel phenotypes that included reduced tassel length without branches and empty spikelets, suggesting that *spi1* and *eos1* function in different pathways related to auxin. *eos1*;*bif2* double mutants also looked more severe compared to either *eos1* or *bif2*. Furthermore, mutant *bif2* tassels in a heterozygous *eos1* background were more severe compared to *bif2* tassels. The dosage effect of the *eos1* mutation in *bif2* mutants, indicates a possible physical interaction between EOS1 and BIF2 proteins in coordinating auxin transport which will be tested in the future.

## P120

## Uncovering maize regeneration mechanism through morphogenic regulators mutants

(submitted by Jiaqi Zhou <jizhou@cshl.edu>)
Full Author List: Zhou, Jiaqi[1]; Chen, Zongliang[2]; Iohannes, Sessen[1]; Clark, Theresa[3]; Skopelitis, Tara[1]; Gallavotti, Andrea[2]; Jackson, David[1]
[1] Cold Spring Harbor Laboratory; Cold Spring Harbor, NY 11724
[2] Waksman Institute, Piscataway, NJ 08854
[3] University of Pennsylvania; Philadelphia, PA 19104

Plant regeneration is critical for advancing crop improvement and functional genomic studies. In maize, regeneration relies on availability of immature embryos. Recently, morphogenic regulators have significantly boosted plant regeneration. For example, ectopic overexpression of GROWTH REGULATING FACTOR4-GRF-INTERACTING FACTOR1 (GRF-GIF) and BABYBOOM (BBM) (collectively termed 'GGB' system) markedly enhance maize regeneration efficiency. Notably, GGB leaves produce somatic embryos in tissue culture, a response absent in wild-type maize. However, the mechanisms underlying this leaf-derived somatic embryogenesis remain unclear. To identify key molecular markers in regeneration, we are conducting time-course mRNA-seq on GGB and B104 leaf callus. Differentially expressed genes were fitted across timepoints and clustered according to expression patterns, revealing clusters uniquely expressed in GGB cultures. Specifically, we found a cell wall loosing related enzyme, *xyloglucan endotransglycosylase homolog 1*, significantly upregulated in GGB. In addition, to characterize hormonal and developmental effects in GGB somatic embryogenesis, we are introducing fluorescent reporters for the auxin transporter PINFORMED1 (PIN1-YFP), auxin signaling marker DR5-RFP, and WUSCHEL (WUS-YFP). Assessing these reporter lines using confocal microscopy will enable real-time monitoring of spatio-temporal molecular processes in leaf – derived embryogenesis. Taken together, these strategies will enhance our understanding of maize regeneration mechanisms. As a next step, single-cell RNA-seq will be employed to resolve cell-type-specific regeneration mechanisms. In the long-term, this project seeks to overcome the bottleneck of regeneration in recalcitrant maize genotypes.

Gene / Gene Models described: *BABYBOOM, Xyloglucan endotransglycosylase homolog1*; Zm00001eb144510, Zm00001eb226470
Funding acknowledgement: National Science Foundation (NSF)

## P121

## Uncovering the genes regulating lifespan stalk flexural stiffness

(submitted by Irene Ikiriko <iikiriko@udel.edu>)
Full Author List: Ikiriko, Irene I.[1]; Hostetler, Ashley N.[1 2]; Sparks, Erin E.[1 2 3]
[1] Department of Plant and Soil Sciences, University of Delaware, Newark, DE 19713, USA
[2] Division of Plant Science and Technology, University of Missouri, Columbia, MO 65201, USA
[3] Donald Danforth Plant Science Center, St. Louis, MO 63132, USA

Stalk mechanics are fundamental to maize production, influencing lodging resistance and interaction with microorganisms. While numerous studies have quantified end-of-season stalk flexural stiffness, it is unclear how stalk mechanics change over the lifespan of plants and how biological factors regulate these changes. We have shown that maize stalk flexural stiffness increases rapidly during early development and plateaus at later stages, and the timing of this plateau varies by genotype. We have further shown that these changes are driven by changes in the material properties of the plants (e.g., cell wall components). However, the cellular mechanisms regulating this trajectory are unknown. We have taken targeted and untargeted approaches to identify the genes regulating the trajectory of stalk flexural stiffness. For the targeted approach, we tested the hypothesis that genes encoding plasma membrane-localized MSL proteins are related to sensing and regulating lifespan changes in stalk flexural stiffness. For the untargeted approach, we quantified stalk flexural stiffness in the Intermated B73 × Mo17 (IBM) Recombinant Inbred Line (RIL) population to detect genetic loci associated with stalk flexural stiffness. Together, these approaches will identify candidate genes regulating lifespan stalk flexural stiffness, informing genetic engineering and breeding efforts aimed at developing mechanically resilient crops.

Funding acknowledgement: National Science Foundation (NSF)

## P122

## *lateral rootless2* (*lrs2*) shapes maize root development by regulating polar auxin transport

(submitted by Emma Klaas <eklaas43@gmail.com>)
Full Author List: Klaas, Emma[1]; Scott, Blake[1]; Ensor, Maya[1]; Wilburn, Colin[1]; Awale, Prameela[1]; McSteen, Paula[1]
[1] Division of Biological Sciences, Bond Life Sciences Center, University of Missouri, Columbia, MO, United States 65201

The plant growth hormone auxin is required for root growth and lateral root initiation. In addition to primary and lateral roots, maize has additional root types, including two types of adventitious roots; seminal roots formed during embryo development and crown and brace roots formed from the base of the stem later in development. An auxin maximum is required for initiation of all root types. Formation of the auxin maximum is controlled by auxin efflux transporter PINFORMED (PIN) proteins, which enable the directional movement of auxin. Several mutants in maize are predicted to impact ZmPIN1 localization and have root phenotypes including *lateral rootless2 (lrs2*) and *barren infloresence2* (*bif2*). The *lrs2* mutant has a short primary root, and a reduced number of lateral and seminal roots. By amplifying the cDNA sequence from *lrs2* mutant roots, we confirmed the presence of a Mutator transposon insertion in the mRNA sequence which causes a premature stop codon. The second allele of *lrs2* is predicted to have an insertion close by the first allele. *lrs2* was crossed with the ZmPIN1a-YFP reporter to visualize auxin transport in primary roots. Confocal microscopy of wildtype 4-day old roots showed PIN localization concentrated in the stele whereas *lrs2* mutants had highly dispersed PIN throughout the root tip. Localization of the PIN protein on the plasma membrane is regulated by phosphorylation and vesicular trafficking. We hypothesize that *lrs2* causes a disruption of this pathway leading to irregular PIN localization, and the *lrs2* root phenotype. To further test this hypothesis, we constructed double mutants between *lrs2* and *bif2*. Studying the auxin pathway in the *lrs2* mutant will show how auxin is integral for root development in maize.

Funding acknowledgement: National Science Foundation (NSF)

# P123

## A comparative approach for selecting orthologous candidate genes underlying signal in elemental accumulation genome-wide association studies across multiple species

(submitted by Magdalena Janik <j.magdalena@wustl.edu>)
Full Author List: Janik, Magdalena[1]; Whitt, Lauren[1 6]; Mahood, Elizabeth[1]; Ziegler, Greg[1]; Luebbert, Collin[1]; Gillman, Jason D.[2]; Norton, Gareth J.[3]; Price, Adam H.[3]; Salt, David E.[4]; Dilkes, Brian P.[5]; Baxter, Ivan[1]
[1] Donald Danforth Plant Science Center, Saint Louis, MO
[2] USDA-ARS Plant Genetics Research Unit, Columbia, MO
[3] University of Aberdeen, Aberdeen, UK
[4] University of Nottingham, Nottingham, UK
[5] Purdue University, West Lafayette, IN
[6] University of Missouri, Columbia, MO

Advances in quantitative genetics and high-throughput pipelines have allowed for rapid identification of genomic markers associated with changes in phenotype. However, linking those markers to causal genes is still difficult, as many genes may be linked to one marker. We aimed to improve candidate gene selection by creating a new method that identifies conserved genes underlying GWAS loci in multiple species. Using this method with GWAS measuring elemental uptakes (ionomic) traits, we identified 14,336 candidates across Arabidopsis, soybean, rice, maize, and sorghum. Acquiring and homeostatically maintaining the cellular concentrations of elements are functions shared by all living organisms. Thus, it was not surprising to find that nine ortholog groups linked to GWAS loci across all five species contained the most likely candidate genes according to calculations using random permutations of the data. These 5/5 ortholog groups include seven orthologs in maize and thirteen in sorghum. Two ortholog groups have previously known elemental uptake functions, and the rest contain orthologs suggested to have previously unknown functions. We are verifying these predictions in the Arabidopsis orthologs by measuring ionomic phenotypes of T-DNA mutants and are identifying alleles of orthologs in maize and sorghum for similar mutant screens. Our methods highlight the conserved nature of ionomic genetic regulators and enable the identification of previously unknown ionomic genes. Based on those results we have curated a list of 23 previously unknown Arabidopsis transporter genes of which we are interested in exploring the mechanism of action in vivo.

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

# P124

## A large-scale transcriptomic survey of nitrogen recycling genes in perennial grasses for sustainable maize production

(submitted by Jonathan Ojeda-Rivera <joo29@cornell.edu>)
Full Author List: Ojeda-Rivera, Jonathan O[1]; Oren, Elad[2]; Hsu, Sheng-Kai[1]; Lepak, Nicholas[3]; La, Thuy[1]; Romay, Cinta[1]; Buckler, Edward S[1 3 4]
[1] Institute for Genomic Diversity, Cornell University, Ithaca, NY USA 14853
[2] Newe Ya'ar Research Center, Institute of Plant Sciences, Agricultural Research Organization – Volcani Institute, Ramat Yishay, Israel 3009500
[3] USDA-ARS, Ithaca, NY, USA 14853
[4] Section of Plant Breeding and Genetics, Cornell University, Ithaca, NY USA 14853

The intensive use of nitrogen fertilizers in maize agriculture has severe environmental impacts and imposes substantial economic burdens on farmers. Enhancing nitrogen use efficiency and recycling in maize is essential to address these challenges. However, modern maize breeding has already exploited much of the genetic diversity for traits like nitrogen use efficiency, making further progress reliant on resources outside the conventional germplasm pool. Perennial grasses in the Poaceae family, including maize's wild relatives in the Andropogoneae tribe, have evolved highly efficient nitrogen recycling strategies, offering a valuable genetic resource for improving maize. In this study, we conducted high-throughput, time-course transcriptional profiling of 14 grass species to identify key genes and promoters for nitrogen recycling. Our species set consists of 12 perennial accessions, including energy crops such as switchgrass and silvergrass, as well as the maize wild relatives Tripsacum dactyloides and Zea diploperennis, along with 2 annual accessions: sorghum and maize. Using machine learning and comparative genomic approaches, we analyzed more than 1,500 RNA-seq libraries from aboveground and underground tissues, capturing physiological and developmental transitions in field-grown plants. Our approach enabled the identification of gene and promoter sequences that could facilitate the engineering of traits like nitrogen remobilization to roots at the end of the growing season to recycle nitrogen on farm. Beyond nitrogen recycling, our findings provide valuable insights into perennial strategies that could be leveraged to enhance climate resilience and sustainability in maize agriculture.

Funding acknowledgement: United States Department of Agriculture (USDA)

**P125** @MaizeGDB

## A unified VCF data set from nearly 1,500 diverse maize accessions and resources to explore the genomic landscape of maize

(submitted by Carson Andorf <carson.andorf@usda.gov>)
Full Author List: Andorf, Carson M[1 2]; Grover, Corrinne[3]; Ross-Ibarra, Jeffrey[4]; Seetharam, Arun S.[3]; Hufford, Matthew B.[3]; Woodhouse, Margaret R.[1]
[1] USDA-ARS, Corn Insects and Crop Genetics Research Unit, Ames, IA 50011, USA
[2] Department of Computer Science, Iowa State University, Ames, IA 50011, USA
[3] Department of Ecology, Evolution, and Organismal Biology, Iowa State University, Ames, Iowa, USA, 50011
[4] Department of Evolution and Ecology, Genome Center, and Center for Population Biology, University of California, Davis, CA 95616, USA

Efforts to capture and analyze maize nucleotide diversity have ranged widely in scope, but differences in reference genome version and software algorithms used in these efforts inhibit comparison, and these data are generally not available in an easy-to-use visualization platform for quick access and analysis. To address these issues, the USDA resource the Maize Genetics and Genomics Database (MaizeGDB) collaborated with maize researchers to offer variant data from a diverse set of 1,498 inbred lines, traditional varieties, and teosintes through a standardized variant-calling pipeline against version 5 of the B73 reference genome. MaizeGDB has also updated a web tool, SNPversity 2.0 https://wgs.maizegdb.org/, to filter, visualize, and download genotype sets based on genomic locations and accessions of interest, and added external data sets to demonstrate SNPversity 2.0's broad usage. MaizeGDB plans to host annual updates of these resources as additional resequencing data become available, with plans to expand to all publicly available sequence data.
The initial WGS build was performed for MaizeGDB by the company Gencove https://gencove.com/. To reduce costs, MaizeGDB together with ISU have chosen to generate in-house builds. To facilitate variant discovery within thousands of individuals, we have explored various optimized variant identification pipelines and settled on Sentieon, a resource-managed pipeline similar to GATK. In general, we find that Sentieon scales well with the number of resources provided, averaging ~1 hour per sample for read mapping-to-haplotyping using 100 CPU with easy distribution across nodes. Joint genotyping was similarly swift, averaging ~0.157 minutes per sample when distributing each of the 10 chromosomes across nodes and using 100 CPU each. The optimization gained via Sentieon will allow MaizeGDB to increase the scope of variant discovery among the many resequenced maize lines as information from new germplasm becomes available.

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

**P126** @MaizeGDB

## Using the MaizeGDB genome browser to evaluate gene model annotation quality and gain functional insights

(submitted by Carson Andorf <carson.andorf@usda.gov>)
Full Author List: Woodhouse, Margaret R.[1]; Cannon, Ethalinda K.[1]; Portwood III, John L.[1]; Haley, Olivia C.[1]; Hayford, Rita K.[1]; Cortes-Tibbs, Laura[1]; Gardiner, Jack M.[2]; Lisch, Damon[3 4]; Andorf, Carson M.[1]
[1] USDA-ARS Corn Insects and Crop Genetics Research Unit, Ames, IA 50011, USA
[2] Division of Animal Sciences, University of Missouri, Columbia, MO 65211, USA
[3] Center for Plant Biology; Purdue University, West Lafayette, IN, USA 47907
[4] Department of Botany and Plant Pathology; Purdue University, West Lafayette, IN, USA 47907

MaizeGDB's genome browsers provide a suite of tools, data, and resources to enable users to evaluate gene model annotation quality and gain functional insights into maize genomics. MaizeGDB now has a set of Syntenome tracks for B73 version 5 that offer whole-genome alignments with other grasses and their lifted gene models to enable comparative analyses that support gene model validation and highlight conserved and diverged regions. These tracks include unmethylated regions, aligned protein fragments from the Maize Peptide Atlas, and confidence scores from protein structure predictions. Along with over 300 gene expression tracks, these datasets support a robust framework for both inter-species and cross-species comparisons. Additionally, protein structure alignments from AlphaFold and ESMFold, alongside full proteome alignments from over 20 species via UniProt and Phytozome, lend further validation and functional context. Alternative annotations from NCBI, Helixer, and Mikado offer confirmatory evidence, while transcriptional start site predictions from CAGE data refine the 5' untranslated regions of gene model annotations. Functional insights are further enriched by curated annotations, including UniProt descriptions, Gene Ontology terms, AI-based predictions, and transcription factor annotations from Grassius. Pangenome and pan-gene annotations from the NAM founder genomes and other high-quality maize assemblies enable users to explore structural and functional diversity across maize lineages. Over 600 epigenetic and DNA-binding datasets, such as transcription factor binding sites, open chromatin regions, and histone modifications, add additional layers of regulatory context. The genome browser also integrates tools for visualizing SNPs, INDELs, and large-scale variations, alongside forward genetics resources like UniformMu, BonnMu, and Ac/Ds insertions. Tight integration with gene model pages, BLAST tools, SNPVersity (a variant viewer), and PanEffect (a variant effect viewer) ensures seamless navigation and analysis. Together, these resources allow users to critically evaluate gene models, uncover functional insights, and advance maize genetics and genomics research.

Funding acknowledgement: United States Department of Agriculture (USDA)

## P127

### Updating the maize nomenclature recommendations

(submitted by Ethalinda Cannon <ethy.cannon@usda.gov>)
Full Author List: Cannon, Ethalinda KS[1]; Cryan, Elli[2 3 4]; Kellogg, Elizabeth A[5 6]; Leiboff, Samuel[7]; Strable, Joshua[8]
[1] USDA-ARS, Corn Insects and Crop Genetics Research Unit, Ames, IA 50011, USA
[2] Plant Sciences, UC Davis, Davis, CA
[3] Evolution and Ecology, UC Davis, Davis, CA
[4] Center for Population Biology, UC Davis, Davis, CA
[5] Donald Danforth Plant Science Center, St. Louis, MO
[6] Arnold Arboretum of Harvard University, Boston, MA
[7] Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR 97330, USA
[8] Department of Genetics, Development and Cell Biology, Iowa State University, Ames, IA USA

The naming of things matters. Clear, unambiguous names for biological entities are vital for research. A new committee is forming to address maize nomenclature. The current specification was completed in 1996, with a 2021 addendum for naming genomic entities (genome assemblies, gene model annotations, and gene models). Much has changed in the intervening 30 years: plant genome assemblies have become a reality, including a plethora of assemblies for maize, leading to new marker types and other genomic features; rapid development of powerful bioinformatic analyses and web-based resources; new types of research, and therefore, new entities requiring names; a much larger and more international research community; increased research within and across the *Zea* genus and related genera. In addition to nomenclature recommendations for new entities, the older recommendation should be revisited, although *existing names will not be changed*. Community involvement will be vital in this effort. Please visit the poster and leave comments, or better, consider joining the committee. Contact information will be on the poster.

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## P128

### Maizemine: Tools for mining gene expression in the maize NAM lines

(submitted by Jack Gardiner <jack.m.gardiner@gmail.com>)
Full Author List: Gardiner, Jack, M.[1]; Walsh, Amy, T.[1]; Andorf, Carson, M.[2]; Elsik, Christine, G.[1 3]
[1] Division of Animal Sciences, University of Missouri, Columbia, MO
[2] USDA/ARS/MaizeGDB, Ames, IA,
[3] Division of Plant Science & Technology, University of Missouri, Columbia, MO

Maize researchers need computational tools that allow them to analyze their unpublished data within the context of an ever-increasing collection of published data characterizing the maize genome. To address this need, MaizeGDB (http://maizegdb.org) collaborated with the University of Missouri to create MaizeMine (http://maizemine.maizegdb.org), based on the Intermine (http://intermine.org) data warehousing platform, to provide a collection of web-based tools in addition to an application programing interface (API) for programmatic access. The List Tool allows users to upload identifiers for their data and perform set operations such as union and intersection and to execute a variety of template queries with their lists. In addition, users can also easily compare their results with published results by either uploading genomic coordinates or identifiers. Over 70 pre-constructed Templates within MaizeMine enable users to access complex quires that allow exploration and integration of diverse data sets. Using the Query Builder tool allows advanced MaizeMine users to either modify or create custom templates that meet their exact needs. The Regions Search Tool allows users to search for a variety of genomic features within a user specified genomic region. The MaizeMine Key Word search allows MaizeMine to be searched for identifiers, names or keyword for genes, pathways, authors, or ontology terms. Here we describe the release of MaizeMine v1.6 in June 2024. Significant to this release is the inclusion of 552 tissue-specific RNA expression data sets and metadata generated by MaizeGDB's qTeller, ATAC peak data, and Illumina SNP50 data for each of the 25 NAM lines. New Grassius Transcription Factor and BonnMu insertion data have been added to B73_v5 assembly. Existing data sets from UniProt, KEGG, Reactome, Interpro, and orthologs from Ensembl Plants have all been updated. SNPs at MaizeMine have been reconfigured to include both the European Variation Archive (EVA) SNPs with rsIDs and SNPs from Ensembl Plants with Panzea IDs. New API code to allow programmatic access to the Regions Search Tool has been developed and made available on GitHub. As always, we welcome suggestions for data sets to include in future MaizeMine releases.

Funding acknowledgement: United States Department of Agriculture (USDA)

## P129 @ohaley93

### Comparing the performance of protein folding models AlphaFold, ESMFold, and Boltz for classical genes in maize

(submitted by Olivia Haley <olivia.haley@usda.gov>)
Full Author List: Haley, Olivia C.[1]; Hayford, Rita K.[1]; Woodhouse, Margaret R.[1]; Cannon, Ethalinda K.[1]; Gardiner, Jack M.[2]; Portwood, John L.[1]; Tibbs-Cortes, Laura E.[1]; Andorf, Carson M.[1]
[1] USDA-ARS Corn Insects and Crop Genetics Research Unit, Ames, IA, USA 50011
[2] Division of Animal Sciences, University of Missouri, Columbia, MO, USA 65211

The field of structural biology took a major step forward in 2020 with the advent of AlphaFold - a program that uses deep neural networks to predict the 3D conformation of proteins. Other models harnessing large language models (ESMFold) and diffusion (Boltz-1, AlphaFold3) have since been released, prompting an increasingly contentious discussion on benchmarking in the protein structure space. In plants, proteins are vital for growth, development, stress resilience, and crop productivity, yet the ability of these models to accurately predict their 3D conformations is often underexplored. We assessed the performance of three models – AlphaFold2, ESMFold, and Boltz-1 – for 417 classical genes (historically mapped via Mendelian phenotypes) in Zea mays. On A100 and V100 GPU cores, ESMFold generated structure predictions ~170X faster than AlphaFold2 and ~4X faster than Boltz-1. Structural alignments were performed in Foldseek to determine how well the predicted structures agreed with one another globally (e.g., SCOPe fold) and locally (e.g., per-residue distance) for the 386 gene models that were folded by all three models. Currently, we are exploring whether the predicted structures with low global (TM score

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

## P130 @ohaley93

### Fusarium protein toolkit: An AI-based resource to facilitate exploring host-pathogen interactions in maize

(submitted by Olivia Haley <olivia.haley@usda.gov>)
Full Author List: Haley, Olivia C[1]; Kim, Hye-Seon[2]; Harding, Stephen[2]; Proctor, Robert H.[2]; Woodhouse, Margaret R.[1]; Sen, Taner Z.[3]; Andorf, Carson M.[1]
[1] Corn Insects and Crop Genetics Research Unit, US Department of Agriculture – Agricultural Research Service, Ames, IA, USA 50011
[2] Mycotoxin Prevention and Applied Microbiology Research Unit, US Department of Agriculture – Agricultural Research Service, Peoria, IL, USA 61604
[3] Crop Improvement Genetics Research Unit, US Department of Agriculture – Agricultural Research Service, Albany, CA, USA 94710

In staple crops like maize and wheat, Fusarium continues to pose major risks to food security and safety through devastating plant diseases and the production of harmful mycotoxins. Addressing these threats calls for innovative, genomics-enabled solutions that identify potential targets for effective control. MaizeGDB, in partnership with the USDA Mycotoxin and Applied Microbiology Research Unit, have developed the Fusarium Protein Toolkit (FPT), a comprehensive set of web-based tools that integrates genomic and functional data to facilitate a deeper exploration of the molecular mechanisms underlying Fusarium infections in maize. Through the FPT and other curated resources, MaizeGDB aims to accelerate discovery and inform strategies for mitigating Fusarium-related crop losses and mycotoxin contamination. Using a series of bioinformatics packages, we have predicted nearly 2,000 secreted effector proteins across six Fusarium species, presenting their predicted functions, structures, and subcellular locations in a user-friendly format. We also computed the functional consequences of point mutations in Fusarium proteins, which may reveal residues critical to effector virulence. We predicted 15,700 protein-protein interactions (PPIs) between select F.graminearum effectors and maize proteins, and plan to refine our predictions through experimental validation. FPT also features AlphaFold and ESMFold-generated protein structures from six Fusarium species that are easily accessible through a web portal, and can be used to compare the structure of effector orthologs across the genus. In summary, this toolkit facilitates comparative analysis and functional annotation inference of protein effectors within Fusarium species, and lays the foundation for a workflow to study cross-species interactions in maize, identify potential targets for breeding disease-resistant crops and gain deeper insights into pathogenicity and host defense mechanisms. Future developments will include resources to display PPI networks, and expanding the toolkit based on community feedback to other major pathogens in maize, such as Aspergillus flavus. The Fusarium Protein Toolkit is available at https://fusarium.maizegdb.org.
Funding acknowledgement: United States Department of Agriculture (USDA), Department of Energy (DOE)

# P131

## Plasticity and fitness trade-offs in switchgrass revealed by open science and citizen science data

(submitted by Laura Tibbs-Cortes <laura.tibbs-cortes@usda.gov>)
Full Author List: Tibbs-Cortes, Laura E[1 2]; Jewell, Jeremy[3]; Bartley, Laura[3]; Swaminathan, Kankshita[4]; Andorf, Carson[1 2]; Juenger, Thomas[5]; Yu, Jianming[2]; Li, Xianran[3 6]
[1] USDA-ARS Corn Insects and Crop Genetics Research Unit, Ames, IA, USA 50011
[2] Iowa State University, Ames, IA, USA 50011
[3] Washington State University, Pullman, WA, USA 99164
[4] HudsonAlpha Institute for Biotechnology, Huntsville, AL, USA 35806
[5] University of Texas at Austin, Austin, TX, USA 78712
[6] USDA-ARS Wheat Health, Genetics, and Quality Research Unit, Pullman, WA, USA 99164

Switchgrass (*Panicum virgatum*), a member of the Panicoideae subfamily which includes maize, is a perennial grass native to the North American tallgrass prairie that today is grown as a biomass crop for bioenergy. Switchgrass provides a unique opportunity to study adaptation and phenotypic plasticity across a large environmental gradient because it not only has a wealth of open science genomic and phenotypic data available from multi-environment trials (METs), but is also widely observed by citizen scientists across its extensive native range. Based on thousands of research-grade observations from the citizen science repository iNaturalist, we identified a conserved trend in flowering time across latitudes. We then applied the CERIS-JGRA algorithm and QTL mapping to open science MET data in order to identify specific genetic and environmental factors influencing flowering time as well as biomass and over-winter survival rates. We found that these traits were strongly influenced by temperature and identified three major candidate genes underlying this plastic response to the environment. Candidate genes are currently being validated by CRISPR. Intriguingly, by aligning the switchgrass proteome to the maize proteome, we found that two of these three candidate genes were also recently published as candidate genes underlying plasticity in maize. Alternative haplotypes at these loci differ substantially in their degree of phenotypic plasticity in response to identified environmental cues, resulting in fitness trade-offs across the native range of switchgrass. We combined our CERIS-JGRA models with past weather data and future climate projections to model shifts in the distribution of switchgrass populations over time. Finally, by uncovering and resolving apparently opposite trends in flowering time between the citizen science and MET data, we provide insights for interpretation of these data types while leveraging their complementary strengths.

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## P132

## Alleles that reduce pollen fitness can condition heterogeneous spatial patterns of inheritance across maize ears

(submitted by John Fowler <fowlerjo@oregonstate.edu>)
Full Author List: Ruggiero, Diana[1]; Bang, MIchelle[2]; Leary, Marilyn[1]; Flieg, Harrison[1]; Garcia-Lamas, Luis[2]; Vejlupkova, Zuzana[1]; Megraw, Molly[1]; JIang, Duo[2]; Leiboff, Sam[1]; Fowler, John E[1]
[1] Botany & Plant Pathology Dept; Oregon State University; Corvallis, OR 97331
[2] Statistics Dept; Oregon State University; Corvallis, OR 97331

A greater number of pollen grains often land on recipient flowers than there are ovules to fertilize. Consequently, post-pollination competition, which is influenced by the genotype of the haploid male gametophyte, can lead to deviations from 1:1 Mendelian inheritance. Maize, with its elongated stigma and style (the silk), has a conspicuous spatial heterogeneity, with longer silks at the base of the inflorescence (ear) than those at the apex. Thus, the ear provides an experimental system where the relative distance of pollen tube growth varies predictably, such that competition among pollen tubes could result in systematic spatial effects on progeny ratios – e.g., more slowly growing pollen tubes generating fewer kernels at the base. To test the hypothesis that alleles with reduced pollen fitness influence the spatial distribution of progeny kernels, we developed an updated phenotyping platform that precisely maps mutant *Ds-GFP* kernel genotypes along the ear (EarVision.v2) and a statistical model that evaluates the relationship between kernel position and transmission ratio. In our dataset of 1384 ears representing 58 *Ds-GFP* alleles, none of those with Mendelian inheritance (48/58) showed any significant non-random spatial trend. In contrast, 50% of alleles showing a pollen-specific transmission defect (5/10) exhibited significant spatial effects on kernel ratios. Most notably, an insertion into a gene encoding a putative actin-binding protein, designated *base-to-apex gradient1 (bag1*), conditions a consistent reduced mutant transmission rate at the ear base relative to the apex. Surprisingly, mutant alleles of two other genes can generate the opposite trend, increased mutant transmission toward the ear base; and the two mutant alleles of the sperm-cell specific *gex2* gene are associated with more complex patterns (e.g., highest at both base and apex). We conclude that effects of pollen fitness mutants on the spatial distribution of progeny seed genotypes on an ear appear heterogeneous and relatively common.

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## P133

## Archaeological Bolivian maize genomes suggest Inca cultural expansion augmented maize diversity in South America

(submitted by Huan Chen <chenhua9@msu.edu>)
Full Author List: Chen, Huan[1 2 4]; Baetsen-Young, Amy[3]; Thompson, Addie[2 3]; Day, Brad[1 2 3]; Madzima, Thelma[4]; Wasef, Sally[5]; Casanovas, Claudia R[6]; Lovis, William[7]; Wrobel, Gabriel[7]
[1] Department of Genetics and Genome Sciences, Michigan State University, East Lansing, Michigan 48824, USA.
[2] Graduate Program in Molecular Plant Sciences, Michigan State University, East Lansing, Michigan 48824, USA.
[3] Department of Plant, Soil and Microbial Sciences, Michigan State University, East Lansing, MI 48824, USA.
[4] Department of Plant Biology, Michigan State University, East Lansing, Michigan 48824, USA.
[5] Genomics Research Centre, Queensland University of Technology, 60 Musk Ave, Kelvin Grove, QLD, 4059, Australia.
[6] Universidad Mayor de San Andres, Avenida Villazón 1995 Monoblock Central La Paz Bolivia.
[7] Department of Anthropology, Michigan State University, East Lansing, MI 48824, USA.

Previous archaeological and anthropological studies have demonstrated the myriad of ways that cultural and political systems shape access to food and food preferences. However, few studies have conducted a biocultural analysis linking specific genotypic/phenotypic traits as evidence of cultural selection in ancient contexts. Here, we provide insight into this topic through ancient genome data from Bolivia dating to ~500 BP, included as an offering with the mummified remains of a young girl, including 16 archaeological maize samples spanning at least 5,000 years of evolution, and 226 modern maize samples. Our phylogenetic analysis showed that the archaeological Bolivian maize (aBM) has the closest genetic distance to the archaeological maize from ancient Peru, which in turn shared the most similarities with archaeological Peruvian maize. The genetic differentiation implies that the Inca state aided maize diversity. The ovule development process was selected from modern maize and was compared to archaeological maize; where it indicates the breeding programs aimed at enhancing seed quality and yield in modern maize. Our study provides insights into the complex biocultural role that Inca Empire expansion, including its economic, symbolic and religious cultural practices, may have had in driving the expansion of maize diversity in South America.
Funding acknowledgement: MSU Research Foundation

## P134

## Building a practical haplotype graph from PacBio assemblies for genotype imputation and allele mining in maize

(submitted by Wei-Yun Lai <wl748@cornell.edu>)
Full Author List: Lai, Wei-Yun[1]; Zhai, Jingjing[1]; Casstevens, Terry[1]; Johnson, Lynn[1]; Berthel, Ana[1]; Miller, Zachary[1]; Franco, Jose A. V.[2]; Romay, M. Cinta[1]; Stitzer, Michelle C.[1]; Hsu, Sheng-Kai[1]; Costa-Neto, Germano[1]; Buckler, Edward S.[1 2 3]
[1] Institute for Genomic Diversity, Cornell University, Ithaca, NY, USA 14853.
[2] Section of Plant Breeding and Genetics, Cornell University, Ithaca, NY USA 14853.
[3] USDA-ARS, Robert W. Holley Center for Agriculture and Health, Ithaca, NY, USA 14853.

Shfting the maize growth cycle by planting earlier in the season can lead to better utilization of sunlight and prolonged land coverage, which in turn helps reduce fertilizer inputs and prevent greenhouse gas emissions. Consequently, increasing seedling cold tolerance is a crucial breeding target for maize cultivation. One strategy to help achieve this goal is the exploration of the existing allelic diversity in the global maize population. We hypothesizes that maize lines developed in northern regions and high-altitudes have adaptive alleles for improved fitness in cold conditions, as they have undergone selection for cold tolerance. We make use of 75 high-quality, long-read genome assemblies from diverse global maize accessions, and perform a genome-wide scan to identify signatures of diversifying selection among these accessions. Further, we will explore the functional consequences of identified variants using an evolution-informed machine learning model. Loci identified through these methods are unbiased with respect to the targets of selection. To identify causal variants for cold adaptation, we will conduct an environment-genotype association analysis using measurements of the environment where the lines were developed. Focusing on loci identified as selected and functionally important will reduce false-positives, improving our power to detect directly actionable alleles. This study aims to provide valuable insights for maize crop improvement and global agricultural sustainability.

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

## P135

## Candidate genes underlying a major QTL *qshgd1* causing spontaneous haploid genome doubling in maize A427

(submitted by Yue Liu <yueliu@iastate.edu>)
Full Author List: Liu, Yue[1]; Seetharam, Arun S[2]; Pfeffer, Sarah J[1]; Liu, Peng[3]; Hufford, Matthew B[4]; Lübberstedt, Thomas[1]
[1] Department of Agronomy, Iowa State University, Ames, IA, USA 50011
[2] Rosen Center For Advanced Computing, Purdue University, West Lafayette, IN, USA 47907
[3] Department of Statistics, Iowa State University, Ames, IA, USA 50011
[4] Department of Ecology, Evolution, and Organismal Biology, Iowa State University, Ames, IA, USA 50011

Doubled haploid (DH) technology accelerates plant breeding by generating completely homozygous lines within two generations, saving at least four generations compared to traditional breeding methods, which demand 6-8 generations of selfing. However, DH technology relies on artificial genome doubling for fertility, involving mutagenic reagents (often colchicine) and related nurseries, presenting both hazardous and labor-intensive challenges. Spontaneous Haploid Genome Doubling (SHGD) is a promising alternative to artificial genome doubling that sidesteps toxic reagents and significantly reduces costs. Our previous work identified a maize genotype A427 for its high occurrence of SHGD. Notably, 78% of A427 haploids exhibit the ability to produce pollen, a stark contrast to the near-zero SHGD rates in most maize genotypes. Mapping experiments have located the major quantitative trait loci *qshgd1* responsible for SHGD in A427 to a 20 Mb region on chromosome 5. However, map-based gene isolation approaches proved challenging, as recombination is suppressed within the 20 Mb region. Here, we report the genome sequence and transcriptome of A427, establishing the gene models specific to A427 and providing a genomic map for expression analysis. Promising candidate genes underlying *qshgd1* have been identified for further investigation based on 1) presence and absence variation between A427 and other non-SHGD genotypes within the chromosome 5 region, and 2) differentially expressed genes between ploidy levels of A427 (haploid vs. diploid) and the non-SHGD genotype Wf9 within the chromosome 5 region.

Funding acknowledgement: United States Department of Agriculture (USDA)

# P136

## Characterization of allele-specific expression and *cis*-regulatory divergence in hybrid maize: Implications for gene-environment interactions

(submitted by Sontosh Deb <skdeb@ncsu.edu>)
Full Author List: Deb, Sontosh K[1 2]; Thomas, Ty S[1 2]; Torres-Rodríguez, J. Vladimir[3 4 5]; Turkus, Jon[3 4 5]; Sekhon, Rajandeep S[6]; Wallace, Jason G[7 8]; Washburn, Jacob D[9]; Thompson, Addie M[10 11]; Schnable, James C[3 4 5]; Gage, Joseph L[1 2]
[1] Department of Crop and Soil Sciences, North Carolina State University, Raleigh, NC 27695, USA
[2] NC Plant Sciences Initiative, North Carolina State University, Raleigh, NC, 27606, USA
[3] Quantitative Life Sciences Initiative, University of Nebraska-Lincoln, Lincoln, NE, 68588, USA
[4] Center for Plant Science Innovation, University of Nebraska-Lincoln, Lincoln, NE, 68588, USA
[5] Department of Agronomy and Horticulture, University of Nebraska-Lincoln, Lincoln, NE, 68588, USA
[6] Department of Genetics and Biochemistry, Clemson University, Clemson, SC 29634, USA
[7] Institute of Plant Breeding, Genetics, and Genomics, University of Georgia, Athens, GA 30602, USA
[8] Department of Crop and Soil Science, University of Georgia, Athens, GA 30602, USA
[9] USDA-ARS, MWA-PGRU, 302-A Curtis Hall, University of Missouri, Columbia, MO 65211, USA
[10] Department of Plant, Soil & Microbial Sciences, Michigan State University, East Lansing, MI 48824, USA
[11] Plant Resilience Institute, Michigan State University, East Lansing, MI 48824, USA

Gene-environment interaction (GxE) is thought to play a major role in determining molecular and organismal phenotypes and adaptive evolution. Although existing empirical evidence suggests that GxE is widespread across organisms and underlies many complex traits, the mechanisms responsible for GxE are not well characterized. Allelic differences in DNA sequences within populations can cause changes in gene expression and regulation. Quantifying allele-specific expression (ASE) by measuring the relative mRNA abundance of each allele at heterozygous loci can serve as a method to characterize whether the regulatory divergence contributes to gene expression GxE. Here, we sought to identify patterns of allele-specific expression divergence across different environments and characterize the contribution of *cis*-regulatory divergence in the allelic sequences to gene expression GxE. We used an existing ASE analysis pipeline with imputed SNP data and RNA-Seq data to identify allele-specific reads and quantify the ASE of each sample at the gene level. We evaluated the performance of this pipeline using a publicly available RNA-Seq dataset of B73×Mo17 hybrids generated from a controlled heat and cold stress experiment and benchmarked our results against published ASE results for the same dataset. Next, we employed the same pipeline to assess the population-level ASE differences in a population of maize hybrids grown in six field environments to characterize the potential contribution of *cis*-regulatory divergence to GxE. We used RNA-seq data of 1,518 individuals of 492 maize hybrids grown in six unique locations across the United States collected through the Genomes to Fields Initiative. Our study provides insights into the effects of allelic sequence differences on GxE in complex real-world environments and shows how GxE potentially manifests into phenotypic variation.

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

## P137

## Cross-species modeling of plant genomes at single nucleotide resolution using a pre-trained DNA language model

(submitted by Jingjing Zhai <jz963@cornell.edu>)
Full Author List: Zhai, Jingjing[1]; Gokaslan, Aaron[2]; Schiff, Yair[2]; Berthel, Ana[1]; Liu, Zong-Yan[3]; Lai, Wei-Yun[1]; Miller, Zachary[1]; Scheben, Armin[5]; Stitzer, Michelle[1]; Romay, Cinta[1]; Buckler, Edward[1 3 4]; Kuleshov, Volodymyr[2]
[1] Institute for Genomic Diversity, Cornell University, Ithaca, NY USA 14853
[2] Department of Computer Science, Cornell University, Ithaca, NY, USA 14853
[3] Section of Plant Breeding and Genetics, Cornell University, Ithaca, NY USA 14853
[4] USDA-ARS; Ithaca, NY, USA 14853
[5] Simons Center for Quantitative Biology, Cold Spring Harbor Laboratory, 1 Bungtown Road, Cold Spring Harbor, NY USA 11724

Interpreting function and fitness effects in diverse plant genomes requires transferable machine learning models. Language models (LMs) pre-trained on large-scale unlabeled biological sequences can learn evolutionary conservation. With fine-tuning on limited labeled data, these models offer superior cross-species prediction compared to supervised approaches. Here, we introduce PlantCaduceus, a DNA LM built on the Caduceus and Mamba architectures, pre-trained on 16 angiosperm genomes spanning 160 million years of divergence. Using a masked language modeling framework, where each nucleotide is treated as a "word," PlantCaduceus captures evolutionarily constrained sequence patterns and achieves robust predictive performance with minimal labeled data. When fine-tuned on Arabidopsis for four key annotation tasks, PlantCaduceus demonstrates high transferability across divergent plant species. These tasks include translation initiation and termination site (TIS/TTS) prediction and splice donor/acceptor site identification, where it outperforms existing DNA LMs by up to 7.23-fold. For example, in maize TIS prediction, it achieves a PRAUC of 0.815, far exceeding the best baseline model's 0.089. In identifying deleterious mutations, PlantCaduceus matches the performance of state-of-the-art protein LMs and surpasses phylogenetic approaches (PhyloP/phastCons) by up to threefold without fine-tuning. Additionally, it successfully identifies known causal variants in both Arabidopsis and maize. Together, these findings highlight PlantCaduceus as a versatile and powerful foundation model with broad utility for plant genomic annotation and crop improvement.

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

## P138 @Yan_Geneticist

### Dancing canopy: Genes regulate azimuthal canopy orientations and response to high planting density in maize

(submitted by Yan Zhou <yzhou86@iastate.edu>)
Full Author List: Zhou, Yan[1 2]; Kusmec, Aaron[1 3]; Hickman, Jesse[1 4]; Schnable, Patrick S.[1 2]
[1] Department of Agronomy, Iowa State University, Ames, Iowa 50011, USA
[2] Plant Science Institute, Iowa State University, Ames, Iowa 50011, USA
[3] Current address: Department of Agronomy, Kansas State University, Manhattan, Kansas 66506, USA
[4] Current address: Department of Forestry and Environmental Resources, North Carolina State University, Raleigh, North Carolina 27695, USA

Light interception is a function of canopy architecture, including, plant density, leaf number, length, width, and angle, as well as azimuthal canopy orientation. Previously, we conducted genome-wide association studies (GWAS) on the parallel canopy trait and the fraction of photosynthetically active radiation (PAR) intercepted by canopies and thereby identified candidate genes, many of which are associated with shade avoidance syndrome (SAS) and leaf development, such as *phytochromeC2* (*phyC2*) and *liguleless1* (*lg1*). Here we report that the altered azimuthal canopy orientations of *Mu*-insertion mutants of tested GWAS candidates are a response to higher planting densities. Under high planting density, mutants of SAS and *liguleless* genes (*lg1* and *lg2)* exhibit altered canopy patterns, viz., leaves are reoriented towards the axis of planting as compared to non-mutant controls. Yet no significant differences were observed under low density. Further, the results of our transcriptome analyses on emerging leaves from plants grown under low and high planting densities provide further evidence that *liguleless* gene functions are required for normal light responses. These analyses also support our model that azimuth canopy re-orientation is achieved via interactions between red/far-red light-induced SAS and blue light-mediated phototropism.

Gene / Gene Models described: *lg1*, *lg2*, *phyC2*; GRMZM2G036297, GRMZM2G060216, GRMZM2G129889
Funding acknowledgement: National Science Foundation (NSF)

## P139

### Decoding tissue-specific gene expression using DNA language model

(submitted by Szu-Ping Chen <sc3367@cornell.edu>)
Full Author List: Chen, Szu-Ping[1]; Zhai, Jingjing[2]; Buckler, Edward S.[1 2 3]
[1] Section of Plant Breeding and Genetics, Cornell University, Ithaca, NY USA 14853
[2] Institute for Genomic Diversity, Cornell University, Ithaca, NY USA 14853
[3] USDA-ARS; Ithaca, NY, USA 14853

Tissue-specific gene expression is fundamental to cellular specialization and function in plants, where it governs critical processes such as growth, development, and stress responses. Understanding the regulatory mechanisms driving tissue-specific expression can provide insights into maize biology and enhance strategies for crop improvement. However, predicting these expression patterns from DNA sequences remains a significant challenge due to the complexity of gene regulatory grammar and the vast genomic diversity of maize. DNA language models, pre-trained on massive unlabeled datasets, can be fine-tuned for diverse downstream tasks. We previously introduced PlantCaduceus, a novel DNA language model pre-trained on 16 Angiosperm genomes. By using masked language modeling strategy, PlantCaduceus learned the deep conservation and DNA sequence grammar of Angiosperm genomes. Here, we propose to fine-tune PlantCaduceus to predict tissue-specific gene expression by capturing sequence patterns and regulatory motifs critical for differential expression across tissues. We will apply this model to annotated datasets of tissue-specific gene expression to identify key regulatory motifs and sequence patterns embedded in maize genomic sequences that are associated with key agronomic traits. The predicted results can be validated using ATAC-seq data and further applied to 26 NAM lines. This research underscores the importance of leveraging machine learning for unraveling the complexities of gene regulation in maize. Results gained from this approach could pave the way for designing tissue-specific expression systems, improving crop productivity, and enhancing stress tolerance in maize.

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

# P140

## Dissecting the physiological and molecular responses to nitrogen stress in sorghum

(submitted by Jaspreet Sandhu <jsandhu@danforthcenter.org>)
Full Author List: Sandhu, Jaspreet[1]; Kumar, Indrajit[1]; Braud, Max[1]; Sonawane, Balasaheb V[2]; Schachtman, Daniel[3]; Cousins, Asaph[2]; Eveland, Andrea L.[1]
[1] Donald Danforth Plant Science Center, St. Louis, MO, USA
[2] School of Biological Sciences, Washington State University, Pullman, WA, USA
[3] Department of Agronomy and Horticulture, Center for Plant Science Innovation, University of Nebraska-Lincoln, Lincoln, Nebraska, USA.

The current agricultural industry is heavily dependent on nitrogen (N) obtained from synthetic fertilizers, but their excessive use in an N-inefficient crop system is unsustainable due to high economic costs and harmful impacts on the environment. Sorghum, a C4 crop, is naturally adapted to arid and low-nutrient conditions and so is emerging as a candidate bioenergy feedstock for cultivation on marginal US land. This will require further improving N use efficiency (NUE) in sorghum. To dissect the genetic regulation of NUE in sorghum, we conducted a controlled-environment phenotyping experiment where multi-omics analyses were used to investigate sorghum's molecular and physiological responses to limited N in three diverse genotypes (one grain and two bioenergy cultivars). All three showed significant reduction in root and shoot biomass in response to low N compared to sufficient N, however there was variation among genotypes including in root to shoot ratios. We integrated temporal transcriptome and accessible chromatin data from roots and shoots in both N conditions and identified a core set of N-responsive genes. N limitation also altered expression of genes involved in phosphorus (P) starvation, suggesting interdependence and coordinated signaling between pathways regulating N and P utilization. Weighted Gene Co-expression Network Analysis identified co-expressed gene modules significantly associated with C:N elemental ratio and enriched for photosynthetic genes that were strongly suppressed under low N, suggesting that N limitation impacts central carbon metabolism. Interestingly, one of the bioenergy lines showed higher expression of C4 isoforms of NADP-ME photosynthetic pathway genes under both N conditions. Our ongoing work focuses on identifying regulatory regions and transcription factors that regulate the expression of these photosynthetic genes under N stress. Our research provides insights into the molecular mechanisms underlying N stress response in sorghum, which can be utilized in breeding and genome editing strategies for developing N-efficient sorghum cultivars.

Funding acknowledgement: Department of Energy (DOE)

## P141

## Dynamic patterns of maize phenotypic responses to drought, heat, and their combined stress

(submitted by Alejandra Quinones <quino070@umn.edu>)
Full Author List: Quinones, Alejandra[1]; Casto, Anna[2]; Lima, Leonardo W.[2]; Murphy, Katie M.[2]; Gehan, Malia A.[2]; Hirsch, Cory D.[1]
[1] Department of Plant Pathology, University of Minnesota, Saint Paul, MN 55108
[2] Donald Danforth Plant Science Center, St. Louis, MO 63132

Climate change is intensifying environmental stresses, threatening global maize production. To develop stress-tolerant germplasm, it is crucial to understand plant responses to both individual and combined abiotic stresses. This study evaluated the phenotypic responses of 47 diverse maize inbred lines, including the Nested Association Mapping (NAM) panel founders, to drought, heat, and their combination during early vegetative growth. Drought stress was induced by gradually reducing soil water content from 88% to 40%, heat stress by maintaining day/night temperatures of 38/28°C, and combined stress by applying both simultaneously. The high-throughput phenotyping platform in the Bellwether Phenotyping Facility at the Donald Danforth Plant Science Center was used to capture daily high-definition images of the plants, from which morphological and color traits were extracted using computer vision tools in PlantCV. Our results demonstrated significant impacts of all three stress conditions on plant size and color. Notably, responses varied widely among genotypes, independent of subpopulation or their phenotype in control conditions. While many genotypes showed developmental delays under stress, several progressed to similar developmental stages under heat stress as in control conditions despite reduced size. Phenotypic plasticity analysis revealed that the impact of the combined stress was primarily equivalent to the sum of the individual stresses (additive) or more severe than both of them (synergistic). However, some genotypes showed comparable responses to combined stress and individual heat stress, suggesting potential predictability of combined stress responses from heat responses alone. This study provides a foundation for identifying germplasm with tolerance to individual and multifactorial abiotic stresses, advancing efforts to enhance maize resilience to climate change and serving as a basis for future genetic studies into mechanisms of stress tolerance.

Funding acknowledgement: United States Department of Agriculture (USDA)

## P142

## Expanding maize breeding resources with teosinte alleles through a practical haplotype graph map of the Zea Synthetic population

(submitted by Micah Kelleher <mkelleher@danforthcenter.org>)
Full Author List: Kelleher, Micah[1]; Kosfeld, Tim[1]; Ziegler, Greg[1]; Luebbert, Collin[1]; Stetter, Markus[2]; Ross-Ibarra, Jeffrey[3]; Flint-Garcia, Sherry[4]; Baxter, Ivan[1]
[1] Donald Danforth Plant Science Center; St. Louis, MO 63132
[2] Institute for Plant Sciences; University of Cologne; Cologne, Germany 50674
[3] Department of Evolution and Ecology; University of California, Davis; Davis, CA, USA 95616
[4] USDA-ARS; Plant Genetics Research Unit; Columbia, MO, USA 65211

Due to domestication, adaptation, and inbreeding, modern maize cultivars have lower genetic diversity than their wild ancestor, teosinte. Because teosinte offers genetic variation, reintroducing its alleles into maize breeding efforts could be valuable. However, the limited availability of high-resolution genetic resources for teosinte has hindered efforts to link this variation to agronomic traits. We have developed a genetic map of the Zea Synthetic population, a genetic resource created by randomly mating 38 maize lines. This synthetic breeding population consists of 11 geographically diverse teosinte accessions (Zea mays ssp. parviglumis), B73, Mo17, and the 25 Nested Association Mapping (NAM) founders. After six generations of random mating, approximately 2,000 double haploid (DH) lines were produced, originally to study inbreeding depression. Our approach uses low-coverage, genotype-by-sequencing (GBS) reads from the DH lines along with the Practical Haplotype Graph (PHG), a graph-based pangenome tool optimized for plant breeding and genetics, to impute genotypes. The PHG uses consecutive genic and intergenic regions from a reference assembly to generate haplotypes for parental lines based on genome alignments. We populated our PHG with high-quality references for the inbred parental lines and teosinte pseudo-genomes, generated from whole-genome sequences aligned to the B73 reference. GBS reads mapped to this pangenome enabled accurate genotype imputation at high resolution. The genetic map we produced identified known QTL associations for traits such as flowering time and cadmium accumulation, as well as regions of segregation distortion and those that have experienced selection. Ongoing refinements to this PHG pipeline focus on improving imputation accuracy and expanding trait mapping capabilities.

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

## P143

## Exploring the influence of nitrogen on rhizosphere and apoplastic microbiomes in maize

(submitted by Riley Harper <riley.harper@ufl.edu>)
Full Author List: Harper, Riley M.[1]; Khalid, Iman[1]; Ou, Mark[1]; Schuster, Layla A.[1]; Katoch, Karun[2]; Sharma, Lakesh K.[2]; Zhao, Meixia[1]
[1] Department of Microbiology and Cell Science; University of Florida; Gainesville, Florida 32611
[2] Department of Soil, Water, and Ecosystem Sciences; University of Florida; Gainesville, Florida 32611

Applying nitrogen to soil has been shown to impact the microbial diversity of the rhizosphere and apoplastic fluid of maize through affecting the abundance of certain bacterial genera. In this study, we investigate how varying levels of nitrogen concentrations affect microbial communities and the growth and stability of maize over time. DNA was isolated from both the rhizosphere and apoplastic fluid of maize plants treated with different levels of nitrogen concentrations, both before and after flowering. We performed 16S rDNA sequencing to assess bacteria composition and abundance and their relationships to nitrogen levels. Preliminary results reveal distinct bacterial species in the rhizosphere compared to the apoplastic fluid, with DNA concentrations varying between these regions. Differential abundance analysis indicated that bacterial genera such as Nitrosospira and Enterobacter bacteria are negatively correlated with nitrogen levels, showing reduced abundance in samples treated with higher nitrogen concentrations. In contrast, other bacterial families, including Allorhizobium and Leadbetterella, exhibited enhanced abundance in response to higher nitrogen levels. These findings illustrate how elevated nitrogen concentrations influence the composition of bacterial taxa in maize, promoting plant growth and stability through the enrichment of beneficial microbial communities. Our next step is to compare these bacterial communities across two time points and different maize tissues to further elucidate the relationship between microbial diversity and maize growth under varying nitrogen conditions.

Funding acknowledgement: University of Florida Startup funds

# P144

## Genomic assembly and analysis of fast-flowering mini-maize

(submitted by Mohamed El-Walid <mze3@cornell.edu>)
Full Author List: El-Walid, Mohamed Z.[1]; Romay, M. Cinta[2]; Lepak, Nicholas K.[3]; La, Thuy[2]; Buckler, Edward S.[1 2 3]; Birchler, James A.[4]; Stitzer, Michelle C.[2]
[1] School of Integrative Plant Sciences, Section of Plant Breeding and Genetics, Cornell University, Ithaca, NY USA 14853
[2] Institute for Genomic Diversity, Cornell University, Ithaca, NY USA 14853
[3] USDA-ARS; Ithaca, NY, USA 14853
[4] Division of Biological Sciences, University of Missouri, 117 Tucker Hall, Columbia, 65211, Missouri, USA

Transformation of maize is traditionally both costly and time-consuming and could benefit from a system that allows for rapid testing of genetic modification. Fast-Flowering Mini-Maize (FFMM) was developed to be short in stature and rapidly flowering, achieving a ~60-day seed-to-seed cycle. Recent advances in FFMM transformation methods make the rapid generation of genetic modifications in a maize system feasible, and paired with the short stature, those modifications are readily testable in growth chambers and other controlled environments. To facilitate construct design, FFMM transformation, and to better understand the genetic background and underlying sequence variation, we generated PacBio HiFi sequences and assemblies for both FFMM-A and FFMM-B. Our assemblies yielded highly complete genomes, showing the presence of 99.6% of Eukaryotic BUSCO genes, and were scaffolded to chromosomes. Preliminary analyses of assembly quality and contiguity reveal structural rearrangements that appear to contribute to early flowering. We also observe a roughly 1.64% genome-wide sequence divergence between ZmB73V5 and FFMM-A. We hypothesize that the strong selection on early flowering and plant height during the generation of FFMM swept many linked deleterious alleles to fixation. We are testing this using a combination of approaches, including machine-learning-based burden scores of variant effects and measuring alterations to gene expression networks. These high-resolution assemblies, coupled with ongoing transformation advances in FFMM, provide a platform for rapid, cost-effective testing of genetic modifications.

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

## P145

### Gramene plants: Empowering agricultural research from genomic diversity through pan-genome analysis

(submitted by Zhenyuan Lu <luj@cshl.edu>)
Full Author List: Lu, Zhenyuan[1]; Chougule, Kapeel[1]; Kumar, Vivek[1]; Kumari, Sunita[1]; Olson, Andrew[1]; Tello-Ruiz, Marcela K.[1]; Wei, Sharon[1]; Van Buren, Peter[1]; Gladman, Nicholas[1 2]; Ware, Doreen[1 2]
[1] Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724, USA
[2] U. S. Department of Agriculture, Agricultural Research Service, NEA Robert W. Holley Center for Agriculture and Health, Cornell University, Ithaca, NY 14853, USA

Enhancing crop research and improvement hinges on providing agricultural scientists and plant breeders with comprehensive data and advanced tools to develop robust, high-yielding crop varieties. Comparative genomics and integrated datasets from diverse plant genomes enable the discovery of critical genetic insights. The Gramene Plants database (https://www.gramene.org) supports these efforts by offering access to biological data from 150 plant genomes, spanning major crops and model species, and facilitating cross-species genomic applications. Gramene fosters collaboration within the scientific community by promoting data sharing, maintaining high-quality gene annotations, and adhering to FAIR principles through computational innovations and community-driven curation. Recent updates to Gramene include pan-genome resources for key crops such as maize (maize-pangenome.gramene.org/), rice (oryza.gramene.org/), sorghum (sorghumbase.org/), and grapevine (vitis.gramene.org/). These platforms enable researchers to explore core and accessory genomes, investigate structural variants, and identify presence-absence variations (PAVs) and other genome-specific traits essential for breeding and evolutionary studies. The integration of Reference SNP cluster IDs (rsIDs) facilitates linking genetic traits to variants mapped across multiple genomes, enhancing the utility of pan-genome resources. Gramene also offers advanced tools through the Ensembl genome browser, enabling analyses of gene structure, expression, homology, and metabolic pathways. Collaborations with platforms such as EMBL-EBI Expression Atlas, Bio-Analytic Resources (BAR), and the CLIMTools suite further augment its capabilities. For example, BAR's eFP Browsers visualize gene expression in crops like maize, Arabidopsis, soybean, and sorghum, while the CLIMTools suite supports genotype-environment (GxE) association studies. By incorporating over 400 climate descriptors from satellite data, CLIMTools enables genome-wide association analyses that inform breeding strategies. These innovations reaffirm Gramene's role as a vital resource for agricultural genomics, driving collaborative breakthroughs and empowering sustainable crop improvement. The project is supported by USDA ARS (USDA-ARS 8062-21000-051-00D).

Funding acknowledgement: United States Department of Agriculture (USDA)

## P146

### High-throughput phenotyping with high resolution maize ear and kernel imaging

(submitted by Jacob Washburn <jacob.washburn@usda.gov>)
Full Author List: Bloch, Henry[1]; LaFond, Harper[2]; Draves, Melissa A.[3]; Miller, Nathan[4]; Washburn, Jacob D.[2]
[1] Electrical Engineering and Computer Science, University of Missouri, Columbia, MO 65211
[2] USDA-ARS Plant Genetics Research Unit, Columbia MO 65211
[3] University of Missouri, Division of Plant Science and Technology, Columbia MO 65211
[4] Department of Botany, University of Wisconsin – Madison, Madison, WI 53706

Maize ear and kernel traits are key indicators of overall yield. They are also critical for understanding quality and nutritional contents. Typical ear phenotyping is time consuming and can have a high degree of subjectivity and error. To increase data collection capacity and improve accuracy we developed a high throughput ear imaging system based on lessons learned from examples in the literature. The system is designed to be relatively low cost, modular, scalable, and have a low barrier to entry. It uses flatbed photo scanners and Raspberry Pi computers to collect and organize high quality image data. The system's module design enables fast plug and play scaling from a single operator to many in order to accommodate the fluctuating nature of undergraduate staffing in academic research settings. It also enables extremely customizable data collection workflows. The system can be run entirely off-line with images transferred via USB, or it can be connected through Wi-Fi or ethernet for automatic image transfer, backup, and organization. Image processing pipelines previously developed by the University of Wisconsin – Madison are used for trait data extraction.

Funding acknowledgement: United States Department of Agriculture (USDA)

## P147 @JensinaDavis

### How much of phenotypic variation across environments is explained by variation in gene expression?

(submitted by Jensina Davis <jdavis132@huskers.unl.edu>)
Full Author List: Davis, Jensina M.[1 2 3]; Turkus, Jonathan[1 2 3]; Newton, Linsey[4]; Thompson, Addie M.[4 5]; Schnable, James C.[1 2 3]
[1] Quantitative Life Sciences Initiative, University of Nebraska-Lincoln; Lincoln, NE, USA 68588
[2] Center for Plant Science Innovation, University of Nebraska-Lincoln; Lincoln, NE, USA 68588
[3] Department of Agronomy and Horticulture, University of Nebraska-Lincoln; Lincoln, NE, USA 68588
[4] Department of Plant Soil and Microbial Sciences, Michigan State University; East Lansing, MI, USA 48824
[5] Plant Resilience Institute, Michigan State University; East Lansing, MI, USA 48824

Organismal phenotypes are determined by the content of the genome, the properties of the environment, and interactions between the two. Changes in the genome can influence the phenotype of an organism via multiple mechanisms, including changes that alter protein sequences, gene expression levels, transcript splicing, transcript localization, and stop codon readthrough. DNA sequence changes which impact any of these factors can both alter the impact of genotype on phenotype directly, or alter how a given organism will change its phenotype across different environments (i.e. phenotypic plasticity). Using data on gene expression and plant phenotypes (anthesis, silking, plant height, grain yield) collected from 635 genotypes in two environments, we aim to understand the proportion of phenotypic variance which can be explained by genetic factors and how much of this genetically controlled trait variance is explainable by changes in transcript abundance and determine how this varies by environment and for single environment phenotypes, versus multi-environment trait mean versus phenotypic plasticity. We also estimate the relative levels of genomic versus environmental control on the abundance of transcripts and the properties of genes whose transcriptional abundance exhibits greater relative contributions from one factor or the other.

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

## P148

### Impacts of structural variant deletions and InDels on SNP genotype imputation

(submitted by Rommel Jr. Garrido <garri318@umn.edu>)
Full Author List: Garrido, Rommel Jr., M.[1]; Qiu, Yinjie[1 2]; Hirsch, Candice N.[1]
[1] Department of Agronomy and Plant Genetics, University of Minnesota, St. Paul, MN, 55108
[2] Minnesota Supercomputing Institute, University of Minnesota, St. Paul, MN, 55108

SNP data often contains missing genotype information, which is typically imputed before performing downstream analyses. Missing genotype data can arise due to various reasons, such as low sequencing coverage or structural variations (SVs), including deletions in the DNA. In this study, we assessed the impacts of SV deletions and InDels on the accuracy of SNP genotype imputation near and within deleted sequences. Missing SNP genotype data was annotated as being randomly missing or missing due to deleted sequencing using SV deletion and InDel marker information obtained from short-read whole-genome resequencing data. The missing SNP data was imputed using Beagle version 4.1. Genotype probability (GP) and the percentage of correctly imputed SNP genotypes (%Correct) were used to assess imputation accuracy. Our results showed that GP for imputing missing SNP data was significantly lower than that for SNP data that was masked from the original data set. Additionally, the GP and %Correct for masked SNP genotypes outside SV deletion and InDel regions were higher than those within SV deletion and InDel regions. The linkage disequilibrium between SNPs and SV deletion/InDel markers further influences the accuracy of genotype imputation. These findings support our hypothesis that SV deletions and InDels negatively affect the accuracy of SNP genotype imputation. Furthermore, this study provides new insights into the potential impacts of deletions on downstream analyses. Future work will examine the effects of falsely imputed SNP markers within deleted regions on the power to identify marker-trait associations in GWAS studies.

Funding acknowledgement: National Science Foundation (NSF)

## P149

### Incorporation of biological information in deep learning models for phenotypic prediction

(submitted by Daniel Kick <daniel.kick@usda.gov>)
Full Author List: Kick, Danie R[1 2]; Tuyishimire, Esdras[3]; Washburn, Jacob D[1 2]
[1] USDA-ARS, Plant Genetics Research Unit, Columbia, MO, USA
[2] Division of Plant Sciences, University of Missouri, Columbia, MO, USA
[3] Division of Biological Sciences, University of Missouri, Columbia, MO, USA

For phenotypic prediction and genomic selection deep neural networks have received attention as an alternative or complementary method to linear modeling. These models are promising due to their capacity to flexibly incorporate different data types and their demonstrated efficacy in other fields. These models often are challenging to interpret due to their design. Here we consider an approach to deep neural network construction that incorporates biological knowledge into the model and compare performance and interpretations from this approach against neural networks without this constraint and linear models.

Funding acknowledgement: United States Department of Agriculture (USDA)

## P150

### Integration of phenomic, proteomic, and genomic data into a multi-scale network unravels missing heritability for maize response to water deficit

(submitted by Marie-Laure Martin <marie-laure.martin@inrae.fr>)
Full Author List: Djabali, Yacine[1 2 4]; Rincent, Renaud[4]; Blein-Nicolas, Mélisande[4]; Martin, Marie-Laure[1 2 3]
[1] Université Paris-Saclay, CNRS, INRAE, Université Evry, Institute of Plant Sciences Paris-Saclay (IPS2), 91190, Gif sur Yvette, France
[2] Université Paris Cité, Institute of Plant Sciences Paris-Saclay (IPS2), 91190, Gif sur Yvette, France
[3] Université Paris-Saclay, AgroParisTech, INRAE, UMR MIA Paris-Saclay, 91120, Palaiseau, France
[4] Université Paris-Saclay, INRAE, CNRS, AgroParisTech, GQE-Le Moulon, 91190, Gif-Sur-Yvette,

The evolution of maize yields under water deficit conditions is of particular concern in the context of climate change and human population growth. However, the multiplicity and versatility of drought-response mechanisms make the design of new varieties a complex task that would greatly benefit from a better understanding of the genotype-phenotype relationship. The omnigenic model assumes that the entire genome contributes to complex traits, encouraging the consideration of small-effect SNPs that may explain missing heritability. Under such a hypothesis, we implemented here an innovative systems biology approach integrating the genetic determinism of molecular entities through the combination of genome-wide association studies and network inference. At each step of the integration process, a multi-environment mixed model was used to estimate the part of the genotype x water deficit interaction (GxW) variance captured by the genomic regions identified by our method. We applied our approach on a multi-omic dataset, including phenotypic, proteomic, and genomic data acquired from 254 maize hybrids grown under well-watered and water deficit conditions. Our results show that (i) QTLs underlying variations in protein abundance capture a part of the missing heritability of maize response to water stress response; (ii) there exists a synergy between the loci found in the two watering conditions and the loci associated with plasticity indices calculated from the two conditions; (iii) taking this synergy into account in our approach further increases the part of the GxW variance captured.
We found about 400 new loci capturing 89.4%, 66.5%, and 77.5% of the GxW variance of biomass, water use efficiency, and stomatal conductance, respectively, which brings a gain up to 20 points in captured GxW variances. Hence our results show that multi-omics data integration can be an efficient way to capture missing heritability for complex phenotypic traits and identify new candidate genes related to drought response.

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**P151** @Sofiya_arora

## Leveraging natural genetic variation for functional validation of genes with predicted functions in maize

(submitted by Sofiya Arora <sarora3@unl.edu>)
Full Author List: Arora, Sofiya[1]; Cuéllar Perez, Karla M.[1 2]; Shrestha, Nikee[1]; Schnable, James C.[1]; Torres-Rodriguez, J. Vladimir[1]
[1] Center for Plant Science Innovation and Department of Agronomy and Horticulture, University of Nebraska-Lincoln, Lincoln, NE USA
[2] Division of Natural and Exact Sciences, University of Guanajuato, Guanajuato, Mexico

Understanding regulatory relationships between genes plays a critical role in creating descriptive and predictive genotype-to-phenotype models. The gold standard approach to identifying regulatory relationships involves disrupting gene function and profiling the resulting changes in the expression of other genes in mutants relative to wild-type plants. However, this approach is time and labor-intensive, limiting its application to a small number of genes. Here, we evaluate an alternative approach that leverages naturally occurring cis-regulatory variations combined with gene expression data across large diversity panels to identify the downstream targets of genes of interest. We analyzed 230 maize genes with known functions and loss-of-function phenotypes, conducting expression quantitative trait locus (eQTL) analyses for each. In 168 cases (~73%), the gene of interest was associated with a significant cis-eQTL in gene expression data from 693 individuals from the Wisconsin Diversity Panel. Using whole population RNA-seq data we identified significant differentially expressed genes between individuals carrying different alleles of cis-eQTL. To evaluate how well the transcriptional impacts we identify correspond to those that would be identified in the analysis of loss of function alleles, we are currently identifying published RNA-seq datasets evaluating the expression changes associated with the loss of function alleles of these same 168 genes. In a preliminary study of *atg-12*, an autophagy-related gene, a cis-eQTL, was identified at position 220,072,722 on chromosome 5. Individuals with the AA genotype (n = 447) showed a mean *atg12* expression of 98.25 TPM (transcripts per million), 1.3 times higher than the 73.11 TPM observed in the individuals with GG genotype (n = 238). DEG analysis revealed 1,868 upregulated and 2,163 downregulated genes in the GG genotype compared to AA genotype. Notably, several enriched gene ontology categories aligned with findings from a published study comparing wild-type and *atg12* mutant plants in a W22 genetic background.

Gene / Gene Models described: *atg12*; Zm00001eb256700
Funding acknowledgement: United States Department of Agriculture (USDA), Department of Energy (DOE)

## P152

### Localized and systemic nature of metabolic responses in root-mycorrhizae interactions

(submitted by Rohit Kumar <mohank@clemson.edu>)
Full Author List: Kumar, Rohit[1]; Suseela, Vidya[1]; Tharayil, Nishanth[1]
[1] Clemson University, 105 Collings St. 111 BRC, Clemson, SC, USA, 29634

Microbial association are essential components of sustainable agriculture, specifically, arbuscular mycorrhizal fungi (AMF) establish symbiotic relationships with plant roots, triggering both localized and systemic metabolic signatures, which are not fully understood. In this study, we conducted both macro- and micro-scale metabolic mapping in the roots of maize (*Zea mays*) during AMF colonization. Using a global metabolomics approach, a macro-scale comparison was made between the infected and non-infected regions of the infected root, between infected and non-infected roots of the infected plant, and between roots of infected and non-infected plants. At a micro-scale, metabolic signatures within the infected and non-infected regions of the infected roots were spatially mapped at a resolution of 25 µm using targeted mass spectrometry imaging. Multivariate analysis of detected 1,389 global mass features indicated extensive metabolic reprogramming, with distinct clusters of infected primary and lateral roots compared to all other root types. The distinct clusters were driven by the differential expression of 270 mass features in infected and non-infected zones of the root, 452 mass features between infected and non-infected roots of the plant, and 276 mass features between roots of infected and non-infected plants. Annotated mass features were linked to pathways related to defense responses, such as benzoxazinoids, nutrient allocation, and hormonal regulation, suggesting a coordinated root-wide and localized response to AMF colonization. Spatial imaging of 41 specific mass features on the infected root sections indicated that more than half of the mass features exhibited root cell- or region-specific signals, potentially involved in cell-specific interactions of roots with AMF. In contrast, other mass features showing spatial signals throughout the root section likely play a housekeeping role in mediating AMF colonization. These findings provide valuable and novel insights into the localized and systemic metabolic adaptations of maize roots during AMF symbiosis.
Funding acknowledgement: United States Department of Agriculture (USDA)

## P153

### Low-cost, image-based phenotyping of maize abiotic stress responses

(submitted by Katherine Murphy <kmurphy@danforthcenter.org>)
Full Author List: Duenwald, Joseph[1]; Srivastava, Dhiraj[1]; Murphy, Katherine M.[1]
[1] Donald Danforth Plant Science Center, St. Louis, MO 63132

Precise, accurate, and efficient collection of plant phenotypes is an essential step to understanding how maize genetics contributes to morphology, development, and stress responses. Here we describe a low-cost method for capturing plant images, as well as image processing in open-source software PlantCV. Using image-based phenotyping, we collected non-destructive measurements of plant size, shape, and color. Combined with weight-based phenotyping on the DiTech PlantArray system, we further characterized the heat, drought, and combined stress responses of two maize varieties in high-throughput. These phenotyping capabilities are available to both academic and industry researchers through the Phenotyping Core Facility in the Donald Danforth Plant Science Center, which offers equipment rental and comprehensive experimental services.

## P154

### Maize data for biology, breeding, and genomics: The PanMaize Gramene database

(submitted by Doreen Ware <ware@cshl.edu>)
Full Author List: Ware, Doreen[1 2]; Chougule, Kapeel[1]; Kumar, Vivek[1]; Kumari, Sunita[1]; Lu, Zhenyuan[1]; Olson, Andrew[1]; Tello-Ruiz, Marcela K.[1]; Wei, Sharon[1]; Van Buren, Peter[1]; Gladman, Nicholas[1 2]
[1] Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724, USA
[2] U. S. Department of Agriculture, Agricultural Research Service, NEA Robert W. Holley Center for Agriculture and Health, Cornell University, Ithaca, NY 14853, USA

The Gramene project (http://www.gramene.org) has been instrumental in maize genomics, contributing to the sequencing of the first B73 reference and 25 NAM founders. Building on the Gramene and Ensembl infrastructures, we developed crop-specific pan-genome subsites for maize, rice, sorghum, and grape. The maize pan-genome site (https://maize-pangenome.gramene.org), will in its fifth release, feature over 50 maize assemblies, including B73 (three versions), Ab10, teosinte Til11, NAM founders, European flint, and CAAS lines. Each accession has its own genome browser for gene-based queries via text searches or BLAST, with B73 as the reference. The site integrates transcriptomic data across tissues and stages, enabling paralog expression analysis. Maize-centric gene trees support phylogenetic and synteny analyses, linking genes within maize and across species. Gene-centric views provide access to curated literature via GeneRIF, insights into allelic diversity, lineage-specific expansions, and protein homology via amino acid alignments and conserved neighborhoods. The platform includes histone modification and transcription factor ChIP-seq data alongside tissue-specific transcriptomics. Genetic variation data for 1,500 additional accessions will be included in the upcoming release. SNPs are filterable by predicted functional effects, viewable as tables or images, and assigned rsID identifiers to ensure FAIR-compliant data sharing. Community curation tools allow users to flag structural annotation issues and submit gene function updates. Recent advances include a pan-gene index derived from gene trees to improve structural annotation workflows. By fostering collaboration, providing training, and promoting data stewardship, Gramene advances maize genomics and agricultural research. Funded by USDA-ARS-8062-21000-051-00D.

Funding acknowledgement: United States Department of Agriculture (USDA)

## P155

### MatchPlant: An open-source pipeline for UAV-based single-plant detection and data extraction

(submitted by Worasit Sangjan <worasitsangjan.ws@gmail.com>)
Full Author List: Sangjan, Worasit[1]; Pandey, Piyush[1]; Best, Norman B.[1]; Washburn, Jacob D.[1]
[1] USDA-ARS, Plant Genetics Research Unit, Columbia, MO, United States 65211

Efficient detection and evaluation of individual plants is critical for advancing maize genetics and breeding research. This study introduces MatchPlant, an open-source pipeline for single-plant detection using UAV-derived imagery. By combining interactive tools for UAV imagery preparation with automated deep learning methods, including a data extraction module, MatchPlant provides a modular framework adaptable to diverse agricultural practices and research applications. The automated detection leverages the Faster R-CNN object detection model trained on high-resolution undistorted UAV images, effectively removing artifacts common in orthophotos from the training dataset. A developed geospatial transformation method ensures that plant coordinates detected in undistorted images are accurately projected onto orthophotos, enabling reliable spatial analysis and trait extraction. A case study conducted in a maize field demonstrated the pipeline's adaptability, and validation showed that the object detection model achieved 88.4% accuracy on the validation dataset and 85.0% accuracy on the test dataset in identifying individual plants. Additionally, when the detected bounding boxes were projected onto the orthophoto, the method successfully detected 89.8% of the plants, with 87.5% of the projected boundaries closely matching the manually drawn ground truth. Further accuracy improvements are anticipated with more extensive training datasets, and detection and projection method advancements.

Funding acknowledgement: United States Department of Agriculture (USDA), Department of Energy (DOE), Oak Ridge Institute for Science and Education (ORISE)

## P156

## PanvaR: An ergonomic candidate gene discovery workflow in R.

(submitted by Rijan Dhakal <rdhakal@danforthcenter.org>)
Full Author List: Dhakal, Rijan R[1]
[1] Donald Danforth Plant sciences center, Creve Coeur, Mo

Connecting genetic variants to agronomic traits is fundamental to crop improvement programs, with critical applications for enhancing yield, resource efficiency, and stress tolerance. Genome-Wide Association Studies (GWAS) have emerged as a powerful tool for identifying genetic variants associated with phenotypic traits. However, GWAS results are often complicated by linkage disequilibrium (LD), where genetic variants in close proximity show correlated inheritance patterns. This can lead to significant GWAS signals being displaced from the actual causal genetic elements. Here we present PanvaR, an R-based workflow that integrates GWAS, linkage disequilibrium, and variant effect prediction (SnpEff) data to enhance the accuracy of candidate gene discovery. Testing across diverse phenotypes in Sorghum and Setaria demonstrated PanvaR's ability to refine GWAS signals and identify biologically relevant candidate genes. The workflow efficiently narrows down candidate gene lists by incorporating multiple layers of genomic evidence and functional annotations. By systematically integrating multiple sources of genomic evidence, PanvaR provides researchers with contextually enriched candidate gene predictions that can significantly streamline the gene discovery process. This tool addresses a critical need in genetics research for accessible, reproducible candidate gene discovery. As genomic studies expand across species and traits, PanvaR's approach to evidence integration will become increasingly valuable for connecting genotype to phenotype.

Funding acknowledgement: Department of Energy (DOE)

## P157

## Phenobot - A robotic platform for field-based corn plant phenotyping via multi-sensor fusion

(submitted by Aditya Raj <adiraj@iastate.edu>)
Full Author List: Raj, Aditya[1]; Liu, Xuan[1]; Gai, Jingyao[3]; Schnable, Patrick[2]; Yu, Jianming[2]; Tang, Lie[1]
[1] Department of Agricultural and Biosystems Engineering; Iowa State University; Ames; IA; USA 50011
[2] Department of Agronomy; Iowa State University; Ames; IA; USA 50011
[3] Department of Mechanical Engineering; Guangxi University; Nanning; Guangxi; China 530000

High-throughput phenotyping requires the collection of large datasets of corn plant images using a wide variety of sensors, and the characteristics of the sensing platform play a crucial role, including the payload, maximum operation time, and navigability. A battery-powered custom four-wheeled articulated steered robot called Phenobot was built, capable of mounting ten stereo-sensing units to capture the 3D view of the corn plants and a set of navigation sensors to estimate its position. This study aims to navigate the Phenobot autonomously inside the corn rows by fusing data from an RTK GPS receiver, wheel odometry, an IMU (Inertial Measurement Unit), a high-resolution 3D LiDAR (Light Detection and Ranging) sensor capturing under canopy data, and a high-resolution RGB camera. Precise localization of the robot is essential for safe navigation, and this was achieved by fusing sensors in three different stages - local position by fusing IMU and wheel odometry, global position by fusing GPS, and in-row position by fusing 3D LiDAR data, which enables precise environmental perception crucial for localizing robot's position in a semi-structured environment. The problem was simplified to navigating the robot through a narrow corridor with two walls corresponding to adjacent corn rows. The point cloud data from LiDAR was used to fit two parallel planes representing the corn rows separated by a known distance, and the intersection of these two planes with the ground plane enabled us to localize the robot inside the canopy precisely. Post-localization waypoints were estimated parallel to and midway of two plane rows. The Pure Pursuit Control and Model Predictive Control algorithms navigated the robot through these estimated waypoints. To further improve localization accuracy, future work will integrate a 3D point cloud with dense 2D RGB images and pre-train the images to predict the location of corn stalks.

Funding acknowledgement: National Science Foundation (NSF)

## P158

### Predictive modeling of pollen fitness phenotypes from genome scale data identifies expression specificity as a critically informative parameter

(submitted by Sebastian Mueller <Muellese@oregonstate.edu>)
Full Author List: Mueller, Sebastian A. F.[1]; Vejlupkova, Zuzana[1]; Megraw, Molly[1]; Fowler, John E.[1]
[1] Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR 97330, USA

The male gametophyte of flowering plants, primarily visible as pollen, is required for sexual reproduction. It delivers sperm cells to the female gametophyte for double fertilization, which enables the subsequent development of the seed. Due to its haploid nature, mutations that affect pollen function can result in a quantitative phenotypic effect on pollen fitness, detectable when the mutant transmission rate differs from the Mendelian ratio. In maize, a large set of fluorescently-marked insertional mutations, the Ds-GFP lines, provides a resource for measuring the effect of single gene mutations in the gametophyte by determining the ratio of mutant (Green Fluorescent Protein-marked) to wild-type progeny kernels in reciprocal outcrosses – i.e., how each mutation affects pollen fitness. We have developed a machine learning framework that uses expression profiling (e.g., RNA-seq) and genomic feature (e.g., Ka/Ks ratio) data provided by MaizeGDB (https://mfs.maizegdb.org/) to predict which genes significantly contribute to pollen fitness in maize. The framework is based on a pollen fitness dataset derived from measuring mutant transmission rates, using a computer vision pipeline that analyzes maize ear images, for 267 validated Ds-GFP insertions into single genes. Modeling efforts to predict genes with high fitness effects upon mutation (vs. no fitness effect) demonstrate considerable success, attaining auROC values up to 91%. Successful models correctly predict 8/9 pollen fitness mutants previously identified in the literature. Current analyses show that RNA and protein expression data are substantial contributors to predicting the pollen fitness class. Notably, tissue specificity information is the most critical input for achieving strong model performance. Additionally, other genomic features, such as amino acid composition and distribution, Ka/Ks ratio, and measures of synteny can also contribute to well-performing models. Our results suggest that expression data from either RNA-seq or proteomic profiling are among the most information rich sources for predicting phenotype from genome scale data.

Funding acknowledgement: National Science Foundation (NSF)

## P159

### Quantifying transposable element expressions: Utilizing computational biology and comprehensive annotations to recognize various features in RNA-seq data

(submitted by Meera Rajagopal <rajagopal.54@buckeyemail.osu.edu>)
Full Author List: Rajagopal, Meera[1]; Ou, Shujun[1]
[1] The Ohio State University, 281 W Lane Ave, Columbus, Ohio, United States, 43210

Transposable Elements (TEs) are regions within the genomes of organisms that can move around in response to various environmental stressors. Due to their regulatory nature, TEs and the derived RNAs can affect and alter gene expression through various means. The expressions of TEs can be difficult to quantify due to their repetitive nature, the nesting of TE sequences, and their degradation over time. To address this, we worked on methods to improve the recognition of TE expressions from RNA-seq data utilizing tools like subread-align, subread featureCounts, and bedtools. By realigning the initially unmapped reads, creating artificial reference files to highlight features of interest, and adding more annotation features, we could recognize more reads overall. To test the robustness of our approach, we are looking to utilize similar methods but change the primary alignment program and feature assignment. Overall, these methods can help us better understand how TE expressions change when an organism faces different environmental stressors.

## P160 @6zongyan

### ReelGene2: A large language model for single base pair precision gene annotation in diverse plant genomes

(submitted by Zong-Yan Liu <zl843@cornell.edu>)
Full Author List: Liu, Zong-Yan[1]; Berthel, Ana[2]; Gokaslan, Aaron[3]; Kuleshov, Volodymyr[3]; Buckler, Edward S.[1 2 4]; Zhai, Jingjing[2]
[1] School of Integrative Plant Sciences, Section of Plant Breeding and Genetics, Cornell University; Ithaca, NY USA 14853
[2] Institute for Genomic Diversity, Cornell University; Ithaca, NY USA 14853
[3] Department of Computer Science, Cornell University; New York, NY USA 10044
[4] United States Department of Agriculture-Agricultural Research Service; Ithaca, NY USA 14853

Accurate gene structure annotation is critical for interpreting genomic data and understanding biological processes. Existing tools such as Helixer, Tiberius, and SegmentNT face significant limitations when applied to diverse plant genomes. Although Helixer is a highly regarded tool, it struggles with low accuracy in plants. Tiberius underperforms on untranslated region (UTR) predictions, and SegmentNT fails to annotate individual features or output standard GFF formats. To address these challenges, we introduce ReelGene2, a state-of-the-art gene annotation tool built on PlantCaduceus, a pre-trained DNA language model. ReelGene2 combines a novel refinement layer with training on 62 high-quality angiosperm genomes spanning diverse evolutionary lineages, achieving single-base-pair resolution for exons, introns, UTRs, and complex gene architectures. Unlike existing tools, ReelGene2 accurately identifies intricate features, even without RNA evidence, making it particularly valuable for less-studied genomes.Benchmark evaluations demonstrate ReelGene2's superior performance, delivering up to a 10% improvement in precision for exon annotation over Helixer, Tiberius, and SegmentNT. While ReelGene2 directly predicts UTRs, variability across species presents ongoing challenges, limiting current accuracy. To address this, future refinements will focus on higher-quality training data and enhanced modeling of splice junctions and structural boundaries to improve UTR prediction.ReelGene2 provides open access to predicted gene structures, including GFF files for 65 publicly available genomes from Phytozome. These datasets address gaps in existing annotations, which often lack UTR predictions and are biased toward well-studied species. Furthermore, an interactive visualization platform is under development to facilitate exploration and analysis of genomic annotations. By offering precise, scalable, and adaptable predictions across diverse plant genomes, ReelGene2 sets a new standard for gene annotation. It supports advancements in comparative genomics, evolutionary biology, and the broader plant research community, ensuring greater accuracy and completeness of gene structure predictions.

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## P161

### Role of chromatin accessibility in adaptive divergence of duplicate paralogs

(submitted by Sunil Kenchanmane Raju <kenchanmane@gmail.com>)
Full Author List: Murguia, Simoné[1]; Niederhuth, Chad[2]; Kenchanmane-Raju, Sunil[1]
[1] Department of Botany and Plant Sciences, University of Riverside, California, CA
[2] Michigan State University, East Lansing, MI

The retention and variable expression of duplicate paralogs following gene duplication is a key driver of functional innovation in plants. However, the mechanisms by which chromatin accessibility influences paralog expression variability across species and environments remain poorly understood. Maize (*Zea mays*), a staple crop, and its wild relative *Tripsacum dactyloides* share a whole genome duplication event that occurred 5–12 million years ago, post-divergence from sorghum. This shared duplication is hypothesized to have facilitated their adaptation to challenging environmental conditions.We hypothesized that maize and *Tripsacum* employ distinct paralog combinations to mediate growth and stress responses, with chromatin dynamics shaping their differential expression. Through a combination of transcriptomic analyses and chromatin accessibility profiling, we demonstrate that chromatin environment influences paralog expression patterns, particularly in stress conditions. Our findings reveal that *T. dactyloides* exhibits a unique chromatin-mediated response to chilling stress, providing insight into its superior stress tolerance. These results highlight the importance of chromatin accessibility in modulating paralog function, contributing to species-specific adaptations.

Funding acknowledgement: UC Riverside start-up

## P162 @NikeeShrestha2

### Searching for conserved alternate splicing regulation in maize and sorghum

(submitted by Nikee Shrestha <nshrestha5@huskers.unl.edu>)
Full Author List: Shrestha, Nikee[1 2]; Cuellar, Karla[1 2]; Schnable, James C.[1 2]
[1] Center for Plant Science Innovation, University of Nebraska-Lincoln, Lincoln, NE, USA
[2] Department of Agronomy and Horticulture, University of Nebraska-Lincoln, Lincoln, NE, USA

Alternative splicing is a post-transcriptional process that produces more than one transcript isoform from pre-mRNA. Alternative splicing increases protein diversity and in some cases is known to impact phenotype. However, large numbers of splicing variants are observable for many expressed genes, and it can be challenging to distinguish functionally relevant AS events from splicing noise. One way to enrich for functionally relevant alternative splicing events is that these events should be more likely to be conserved across orthologous genes in related species, and more likely to share common sets of regulatory elements. We identified 126,989 conserved exons for 23,519 orthologous gene pairs in maize and sorghum, and quantified variation in alternative splicing across large populations of both maize (n=695 genotypes) and sorghum (n=738 genotypes) using population-scale RNA-seq datasets. GWAS analysis using two methods of quantifying slicing variation -- exon and isoform proportion -- identified significant marker trait associations and both methods tended to give similar results for individual genes. Moving forward we will identify global sets of cis and trans-regulatory variants associated with splicing variation in maize and sorghum and quantify the degree of conservation in splicing regulation between the two species. Additionally, we will test for associations between splicing variation and phenotype variation in both species using a set of flowering time and photosynthetic traits scored across both populations. Findings from our study will help us understand the regulation and function of evolutionarily conserved alternate splicing events and may help improve our understanding of the role of splicing events in determining phenotype in both maize and sorghum.

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

## P163 @R_J_Grove

### Sweet Angle O' Mine: A comparative analysis of leaf angle phenotyping in sorghum

(submitted by Ryleigh Grove <rgrove4@huskers.unl.edu>)
Full Author List: Grove, Ryleigh[1 2]; Tross, Michael[1 2]; Schnable, James[1 2]
[1] Center for Plant Science Innovation and Department of Agronomy and Horticulture, University of Nebraska - Lincoln, Lincoln, NE, United States of America
[2] Complex Biosystems Graduate Program, University of Nebraska - Lincoln, Lincoln, NE, United States of America

Plant architecture, particularly leaf angle, significantly influences light interception, canopy temperature, water use efficiency, and ultimately crop productivity. Traditional methods for measuring leaf angle rely on 2D imaging, which, though offering higher throughput and lower operational costs, are limited in accuracy due to their inability to capture the full geometry of the plant. This investigation aimed to compare the accuracy of 2D imaging and 3D reconstruction techniques for quantifying leaf angles in a population of 366 sorghum plants, with the primary objective being to determine which method provides more accurate and robust leaf angle measurements while considering factors such as throughput, cost, and ease of implementation. Using PlantCV for image segmentation, skeletonization, and leaf insertion angle extraction, here we show the 2D method exhibited a weak and negative correlation (-0.31) with manual measurements, indicating its limited reliability. In contrast, the 3D reconstruction method, which utilized voxel carving algorithms and multiple calibrated 2D images to generate 3D plant models, showed a positive correlation (0.53) with manual measurements, suggesting greater accuracy despite requiring more complex calculations and additional images. While challenges such as segmenting individual leaves within dense canopies and identifying leaf-stem junctions remain for both methods, the 3D approach demonstrated a clear advantage by providing a more robust estimation of leaf angles. By overcoming the limitations of existing methods, this research establishes a framework for developing high-throughput platforms for automated leaf angle phenotyping, enabling more efficient assessments of crop canopy architecture in species with complex canopies, such as maize.

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## P164

## Temporal UAV surveys for robust and accurate maize flowering time prediction

(submitted by Zhongjie Ji <zji7@unl.edu>)
Full Author List: Ji, Zhongjie[1]; Schnable, James[1 2]; Ge, Yufeng[1 3]
[1] Center for Plant Science Innovation, University of Nebraska-Lincoln, Lincoln, NE,68588
[2] Department of Agronomy and Horticulture, University of Nebraska-Lincoln, Lincoln, NE,68588
[3] Department of Biological Systems Engineering, University of Nebraska-Lincoln, Lincoln, NE,68588

The transition from vegetative to reproductive growth represents a critical transition in the life cycle of maize. The timing of flowering time helps to determine adaptation to different environments and flowering time is correlated with variation in a vast number of other phenotypes, from plant height and leaf number to grain yield and photosynthetic parameters. However, in many field studies, flowering time is not scored as accutate scoring of flowering time typically requires researchers to walk the field on daily or bidiurnally. Automated collection or prediction of flowering time data would enable researchers to score this trait across more field experiments, assisting both investigations of the genetic and environmental determinants of flowering time, but also in controlling for the confounding effects of flowering time when studying other traits of interest. Unmanned Aerial Vehicles (UAVs) offer an affordable and flexible phenotyping platform capable of carrying various cameras and sensors to generate diverse and high-quality data. However, it will typically not be practical to fly a UAV over each field each day. Here we assembled and curated a dataset of >200,000 UAV collected images of maize field plots representing diverse genotypes, locations, and growth stages as well as associated labels for flowering time relative to the date of image collection. We use this dataset to train and evaluate a novel flowering time labeling system that enables accurate general-purpose flowering prediction. This method has achieved highly promising results in predicting maize flowering time using only one UAV flight per field site.

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

## P165

## The practical haplotype graph version 2: a streamlined and simple pangenome system

(submitted by Zachary Miller <zrm22@cornell.edu>)
Full Author List: Miller, Zachary R[1 2]; Johnson, Lynn[1]; Casstevens, Terry[1]; Bradbury, Peter[1]; Monier, Brandon[1]; Berthel, Ana[1]; Buckler, Edward S[1 2 3]
[1] Cornell University, Institute For Genomic Diversity, Ithaca, New York, USA
[2] USDA-ARS, Ithaca NY, USA 14853
[3] Cornell University, Section of Plant Breeding and Genetics, Ithaca, New York, USA

The Practical Haplotype Graph (PHG) is a powerful tool for representing diverse plant pangenomes and imputing new sample genotypes regardless of sequencing technology. A trellis graph representing genic and intergenic regions can accurately represent diversity across and between samples. The PHG tool can be used to create custom genomes for alignment, call rare alleles, impute genotypes and efficiently store genomic data from many samples. The initial version of the PHG system (PHGv1) was published in 2022 and addressed many challenges related to alignment, storage and imputation across a pangenome of many diverse genomes. However, after deploying the PHGv1 system in six different plant species, a number of significant opportunities to refine and streamline the PHG platform were identified, which the PHGv2 system attempts to solve. At the core of the PHGv2 system are the dual concepts of a HaplotypeVCF (hVCF) and a Genotype VCF (gVCF). An hVCF is a VCF file for PHG-generated haplotype calls. Limiting these "variants" to 30,000 - 100,000 genic/intergenic regions reduces file size. A gVCF stores base-pair-level variants between a sample assembly and its reference, collapsing matching regions into multi-base Reference Blocks. PHGv2 uses two TileDB databases to store both hVCFs (haplotype data) and gVCFs (SNP data) for downstream processing.
The PHGv2 system also leverages existing standard open-source software like AnchorWave, AGC, bgzip, BCFtools, BioKotlin, ropeBWT3 and standard file formats like VCF, FASTQ and MAF to simplify the inputs and outputs of the Command Line Interface. Special consideration has also been made to ensure the PHGv2 Command Line Interface is intuitive and straightforward for existing and new users while being able to do everything the PHGv1 system can do. Because of this, the PHGv2 system aims to be an easy-to-use, state-of-the-art imputation tool and a useful pangenome representation.

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

## P166

## Tracking the genomic location and chromatin context of meiotic double-strand breaks leading to crossovers

(submitted by Quinn Johnson <qyj2@cornell.edu>)
Full Author List: Johnson, Quinn[1]; Zelkowski, Mateusz[2]; Wheeler, Emily[3]; Thompson, William F.[3]; Hanley-Bowdoin, Linda[3]; Pawlowski, Wojtek P.[1 2]
[1] Plant Breeding and Genetics Graduate Field, Cornell University
[2] Plant Biology Section, Cornell University
[3] Department of Plant and Microbial Biology, North Carolina State University

Recombination events in plants are rare and unevenly distributed along chromosomes, with 20% of maize genes located in regions where crossovers (COs) are suppressed. The ability to control recombination will allow plant breeders to fully utilize variation in these genes. Recombination begins with programmed formation of double-strand breaks (DSBs) that lead to either COs or noncrossovers (NCOs). Although maize experiences ~200-500 DSBs per meiosis, only ~10-20 result in COs. Most DSBs occur in intergenic regions, while most COs occur near genes. We aim to identify the features of the DSBs' local environment that promote CO formation. To do this, we have analyzed high-resolution DSB and CO maps in maize to track the local environment of CO intermediates throughout recombination. We found that the majority of DSBs occur in intergenic regions, but as recombination progresses, CO intermediates are accumulated in genic regions, specifically around gene transcription start and termination sites that exhibit low DNA methylation. To further evaluate whether CO-competent DSBs can be determined based on their genomic context, we examined the timing of DNA replication during S-phase. We noticed that CO hotspot distribution correlates with genome regions replicating early in S-phase. Research suggests that DNA methylation has the greatest effect on CO patterns; however, replication region timing (in early, mid, or late S-phase) is not correlated to DNA methylation context of the DSB and CO sites, indicating that replication timing affects DSB fate independently of the chromatin state. We conclude DSBs located at gene boundaries in hypomethylated areas undergoing DNA replication during early S-phase are more likely to result in COs compared to NCOs. The correlations with DSB local context features will be further validated through a classification machine learning model. Ultimately, understanding how the local context facilitates CO formation will help us design methods to modify where COs occur.

Funding acknowledgement: National Science Foundation (NSF)

## P167

## Transcriptomic analysis of maize heterosis by nitrogen use efficiency

(submitted by Alexandria Tran <tran30@illinois.edu>)
Full Author List: Tran, Alexandria[1]; Li, Catherine HR[1]; Moose, Stephen P[1]; Marshall-Colon, Amy[1]
[1] University of Illinois; Crop Science and Plant Biology; Urbana, IL 61801

In order to maximize heterotic agronomic traits and consistency, maize breeding populations are maintained as inbred plants, then made commercially available as first-generation (F1) crosses of two genetically distinct parents. This hybridization process produces offspring that yield better than their inbred parents through physiological processes whose genetic control is yet to be fully characterized. One well-documented factor in grain yield is plant response to soil nitrogen (N) availability, including shifts in organ nutrient partitioning and development, which are also key components of hybrid vigor. Nitrogen fertilizers are a major input cost for farmers around the world, and their heavy use pollutes local water systems and is a primary contributor to agricultural greenhouse gas emissions. Since one component of hybrid vigor is nitrogen use efficiency heterosis, we have evaluated plants grown in N insufficient and N saturating conditions over the course of the growing season. Heterosis and N use efficiency are complex traits that are difficult to map with standard quantitative genetics due to the numerous functional pathways in which they manifest throughout a diverse population. We take a targeted look at these molecular mechanisms by sampling the genetic backgrounds B73, H99, and the F1 cross of these. Through transcriptomics analysis, we summarize hybrid transgressive gene expression in relation to the differential N treatments. We also leverage the maize pangenome to explore parental allele-specific expression to further interrogate gene regulation patterns within the hybrid plants. With bulk and allele-specific transcriptomes, co-expression networks were created to discover the overlapping and distinct pathways by which hybridization and N response contribute to enhanced grain yield.

Funding acknowledgement: National Science Foundation (NSF)

## P168
## UTR development in maize

(submitted by Colin Finnegan <cpf@iastate.edu>)
Full Author List: Finnegan, Colin P[1]
[1] Iowa State University; Ames, IA, 50014

The maize genome has been characterized sufficiently that it is possible to identify gene phylogeny, down to orphan genes which are unique to maize. This phylogeny gives a timescale to track the development of gene features like the UTR. In this project, machine learning was used to identify sequence features in UTRs that are predictive of their phylogeny and thus reconstruct a timeline of UTR development as the genes they are attached to mature.

## P169 @annusingh1206
## Unlocking sorghum's growth and yield potential: a study on nitrogen regimes and biostimulant applications

(submitted by Anuradha Singh <singha57@msu.edu>)
Full Author List: Singh, Anuradha[1 2]; Newton, Linsey[1]; Thompson, Addie[1 2]
[1] Department of Plant, Soil and Microbial Science, Michigan State University, East Lansing, MI 48824, USA.
[2] Plant Resilience Institute, Michigan State University, East Lansing, MI 48824, USA.

Maintaining crop productivity presents significant challenges due to population growth and climate change. While nitrogen fertilizers enhance grain yield, their excessive use increases production costs and poses significant environmental risks. Biostimulants have been proposed as alternatives, promoting plant growth and performance at lower costs while minimizing environmental impact. This study aimed to evaluate the impact of different nitrogen treatments on field-grown sorghum (*Sorghum bicolor* L.) accessions, including control nitrogen (CN), high nitrogen (HN) with additional side-dressed N, CN supplemented with a microbial inoculant (MI), and CN supplemented with legume-derived protein hydrolysate (PH). Eight representative accessions were phenotyped using both manual and remote sensing methods during vegetative and reproductive growth stages. Results showed significant phenotypic variability across accessions and treatments, with traits related to growth and development such as leaf number, chlorophyll content, photosynthesis, root-shoot architecture, and biomass exhibiting the most pronounced responses during the transition from the vegetative to reproductive stage. Among the treatments, accessions grown under HN exhibited the best performance, followed by those receiving CN+MI. Conversely, the combination of CN and CN+PH resulted in the lowest performance. Furthermore, various remote sensing indices effectively inferred the crop nitrogen status for field-scale nutrient management. Taken together, our findings suggest that integrating microbial inoculants may enhance sorghum productivity while mitigating environmental risks associated with excessive nitrogen fertilization. The next step will involve transcriptomics to elucidate the molecular mechanisms underlying sorghum plant growth responses to different nitrogen and biostimulant treatments, focusing on the involvement of key pathways, hormone signaling/biosynthetic genes, and transcription factors.

Funding acknowledgement: Department of Energy (DOE)

## P170

### Unveiling cold tolerance in maize: Linking chloroplast genomics and environmental adaptation for climate-resilient breeding

(submitted by Henry Dawson <hdd29@cornell.edu>)
Full Author List: Dawson, Henry D.[1]; Hsu, Sheng-Kai[2]; Buckler, Edward S.[1 2 3]
[1] Section of Plant Breeding and Genetics; Cornell University; Ithaca; NY; USA 14853
[2] Institute for Genomic Diversity; Cornell University; Ithaca; NY; USA 14853
[3] Agricultural Research Service; United States Department of Agriculture; Ithaca; NY; USA 14853

Though maize fails to tolerate cold, other grasses endure cold spells, frost, or harsh winters. Addition of this cold tolerance into maize could allow sowing earlier in the season and improve the persistence of the crop through adverse cold events in the spring. This begs the question – what is the genetic basis of cold adaptation across grass species?Chloroplast activity is both vital for growth and sensitive to temperature. We hypothesized that the predictive relationship between bacterial genomic features and their growth conditions will hold between chloroplast genomic features and the environmental conditions of their host plant species. Refseq-quality grass chloroplast genomes were collected from NCBI. We collected enviromic data, including temperature, soil characteristics, precipitation, etc, from environmental databases based on the geographic coordinates of species occurrences in BIEN and GBIF. 240 chloroplast genomes were processed to produce features which were analyzed for correlation or prediction with environmental conditions. This analysis was applied in parallel to bacterial genomes and their growth conditions. We found that the relationship between bacterial genomes and growth conditions is also moderately present between chloroplast genomes and environmental conditions.What this means for breeding maize for cold tolerance is that the chloroplast does display a genomic signal for environmental adaptation and so warrants further large-scale analysis, with the goal of identifying causal genes or variants for adaptation to low temperatures.

## P171

### Using UAV imagery to assess foliar disease resistance

(submitted by Cole Hammett <chhammet@ncsu.edu>)
Full Author List: Hammett, Cole H[1]; Fritz, Katelyn R[2]; Balint - Kurti, Peter J[2]; Gage, Joseph L[1 3]
[1] North Carolina State University Department of Entomology and Plant Pathology, Thomas Hall, 112 Derieux Pl, Raleigh, NC 27607
[2] North Carolina State University Department of Crop and Soil Sciences, Williams Hall, 101 Derieux Pl, Raleigh, NC 27695
[3] USDA - ARS Plant Science Research Unit, USDA Plant Science Research Building, 3127 Ligon St, Raleigh, NC 27606

Southern leaf blight (SLB) is a foliar disease in maize (Zea mays) caused by the necrotrophic fungal pathogen Cochliobolus heterostrophus. Infamous in the United States for the 1970-1971 epidemic, SLB continues to be a major constraint to maize production in tropical and subtropical regions that can be most effectively mitigated through resistant cultivars. Still, field disease assessment relies on manual visual scoring, which can be time-intensive, subjective, and susceptible to observer variability. This research aims to connect high-throughput plant phenotyping to machine learning in order to assess disease severity in diverse maize populations using RGB and multispectral sensors attached to unmanned aerial vehicles (UAVs). During the 2023 and 2024 field seasons, sensor data was collected at an altitude of 10 m and over an area of 10.5 acres (4.2 hectare) of SLB-inoculated single-row plots in total. Machine learning and deep learning techniques will be applied to extract semantic features from these images that are informative of SLB lesions, to predict the severity of SLB disease. To enhance efficiency and robustness, self-supervised learning techniques will be employed, reducing reliance upon breeder-assigned severity scores and improving generalizability across diverse environmental and field conditions. The goal of this project is to develop a UAV-based evaluation model to supplement or replace traditional SLB phenotyping, creating a scalable and transferable approach across different fields and seasons.

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

## P172

## Using the Ds-GFP population to assess the contributions of pollen-expressed genes to maize reproduction

(submitted by John Fowler <fowlerjo@oregonstate.edu>)
Full Author List: Vejlupkova, Zuzana[1]; Leary, Marilyn[1]; Bang, MIchelle[2]; Du, Chunguang[3]; Dooner, Hugo K[4]; Megraw, Molly[1]; Jiang, Duo[2]; Fowler, John E[1]
[1] Botany & Plant Pathology Dept; Oregon State University; Corvallis, OR USA 97331
[2] Statistics Dept; Oregon State University; Corvallis, OR USA 97331
[3] Biology Dept; Montclair State University; Montclair, NJ 07043
[4] Waksman Institute; Rutgers University; Piscataway, NJ 08854

The large set of *Ds-GFP* sequence-indexed insertion alleles, all marked by fluorescent kernels, (Li et al. 2013) provides a valuable resource for elucidating gene function in maize. We have used this resource to measure gene-specific contributions to pollen fitness for >400 insertions, the majority into pollen-expressed genes. In the course of this project, we developed an efficient process to characterize lines from the *Ds-GFP* collection. This includes: selecting for Ac-inactive plants; ensuring that a line contains only one *Ds-GFP* element; and validating the predicted insertion location via PCR and Sanger sequencing. To facilitate validation, we developed DIGIT (DsGFP Insertion Genomic Informational Tool), a pipeline using flanking sequence to map each insertion relative to the appropriate progenitor genome (B73, W22 or A188), followed by automated design of genomic primers for each allele. We found that 69% of ~400 predicted single insertion lines could be validated with a single primer, with a lower percentage (51%, of ~90 lines tested) for lines with two predicted insertion locations. With this extended dataset, we also identified technical errors in predicted insertion location that could be easily addressed, raising the validation rate to 75%. To measure the fitness effect of each insertion on the haploid male gametophyte, ears from reciprocal outcrosses of heterozygous *Ds-GFP* parents were counted to assess mutant (fluorescent) vs. non-mutant kernel segregation. This process was streamlined using the Ear Scanner/EarVision phenotyping pipeline. Application of a Generalized Linear Model framework to the count data, collected over six field seasons, identified at least 37 insertions with significantly reduced transmission through pollen (1 to 47% transmission; fitness costs 0.99 to 0.11). 31 of these have been validated as insertions into pollen-expressed genes, or 11% of the total 289 gene insertions assessed. A MaizeGDB webpage provides information on validated insertions and protocols for *Ds-GFP* characterization.

Funding acknowledgement: National Science Foundation (NSF)

## P173

## Utilizing drone imaging in analysis of genotype by environment in maize

(submitted by Morgan Mathison <mgmb7w@umsystem.edu>)
Full Author List: Mathison, Morgan G[1]; Washburn, Jacob D[2]; LaFond, Harper[2]
[1] University of Missouri Department of Plant Science, Columbia, MO 65211
[2] USDA-ARS Plant Genetics Research Unit, Columbia MO 65211

Environmental conditions directly impact plant phenotypes. While genotypes are a large factor in an organism's makeup, environments can influence expression or suppression of specific genes, causing genetically identical plants to have different phenotypes in separate environments. Although greenhouses are ideal for studying plant phenotypes under highly controlled environmental conditions, they do not reflect the types of conditions farmers face in a growing season. Utilizing drone imaging can address this issue by repeatedly collecting data over a large landscape throughout the growing period to understand the environmental conditions and phenotypic changes in plants. During the summer of 2023, we collected weekly multispectral image data on 460 maize hybrids grown in two unique field locations of the Genomes to Fields (G2F) Genotype by Environment (GxE) project. On-the-ground phenotypes including flowering time, plant height, yield and others were also collected. Images were processed using Pix4D Mapper to create orthomosaics and python scripts were developed and used to calculate vegetative indices including Normalized Differentiation Vegetative Index (NDVI) in order to understand changes in plant health and photosynthesis capacity throughout the season. Future directions will include comparing NDVI across both locations and incorporating genetic, phenotypic, and environmental data to understand the relationships between NDVI and plant growth under different genetic and environmental factors.

Funding acknowledgement: United States Department of Agriculture (USDA)

## P174 @cedjf_

## Within-leaf spatial responses to heat stress reveal varied genetic mechanisms in *Hordeum vulgare*

(submitted by Edward Cedrick Fernandez <edward.fernandez@ndsu.edu>)
Full Author List: Fernandez, Edward Cedrick J[1]; Tu, Gaoya[1]; Bruggeman, Robert S[2]; Liu, Zhaohui[3]; Grzybowski, Marcin[4]; Liang, Zhikai[1]
[1] Department of Plant Sciences, North Dakota State University, Fargo, ND, United States, 58108-6050
[2] Department of Crop and Soil Sciences, Washington State University, Pullman, WA, United States, 99164-6420
[3] Department of Plant Pathology, North Dakota State University, Fargo, ND, United States, 58108-6050
[4] Department of Plant Molecular Ecophysiology, Institute of Plant Experimental Biology and Biotechnology, Faculty of Biology, University of Warsaw, Warsaw, Poland

Global climate warming poses a major challenge to crop production by altering growing conditions, disrupting agricultural systems, and ultimately threatening global food security. Barley (*Hordeum vulgare*), traditionally cultivated in cool temperate regions, thrives under moderate temperatures that optimize its growth and development. As a species adapted to cold climates, it is highly susceptible to elevated temperatures that can severely disrupt its physiological and developmental processes. However, the continuous rise in global temperatures is expected to further amplify these negative impacts. Since heat stress significantly impacts barley by disrupting photosystems and reducing photosynthetic efficiency, we aim to monitor these effects by comparing alterations in photosynthetic traits at the individual leaf level under optimal and heat-stressed conditions. However, the linear-lanceolate shape of barley leaves inherently introduces complexity, as different regions within a single leaf (*i.e.* base, middle, and tip) can exhibit varying sensitivities and adaptive responses to heat stress. This spatial variation adds a layer of complexity to the study and dissection of the genetic mechanisms underlying heat stress responses. By subjecting a subset of the International Barley Core Collection (BCC) to heat and optimal environments, we observed a strong variation of physiological and non-photochemical quenching (NPQ) kinetics traits across the leaf gradient from base to tip of individual leaves. By integrating these findings with existing genomic variants, we uncovered potentially distinct genetic mechanisms driving heat-stress responses in specific leaf segments. Leaf segments from similar conditions were further investigated through transcriptomic analysis, revealing distinct gene sets across segments that could be involved in heat stress regulations. Our findings provide novel perspectives on refining the genetic understanding of heat stress responses and offer valuable strategies for selecting heat-resilient barley, with implications for improving the heat tolerance of other crop species.

Funding acknowledgement: United States Department of Agriculture (USDA)

## P175

## X-ray tomography provides nondestructive multiscale 3D imaging to support maize research

(submitted by Christopher Topp <ctopp@danforthcenter.org>)
Full Author List: Topp, Christopher N[1]; Duncan, Keith E[1]; Lebow, Clara[1]
[1] Donald Danforth Plant Science Center; St. Louis, MO, USA 63132

Using our high resolution industrial scale X-ray tomography (XRT) instrument, we have developed techniques and computational analysis tools to study complex, internal and otherwise hard-to-measure plant structures. An important advantage of XRT is the ability to do nondestructive in situ imaging, particularly for studying root growth in soil and other dynamic processes. By varying the growth medium and pot size, a wide range of root-specific research questions can be addressed without disturbing the roots or removing them from the soil. High throughput imaging of excavated maize root crowns can yield high-content phenotypic data about root system architecture that is not possible using 2D imaging methods. We have also pioneered the use of lab-based X-ray microscopy (XRM) to image intact, complex, and delicate samples in 3D at high resolution. XRM imaging has supported research into root primordium initiation, meristem growth and development, impact of environmental changes on inflorescence architecture, seed biology, and other projects. Correlative imaging is also significantly enhanced by using XRM scan data of whole resin-embedded samples as a road map to guide subsequent nanometer-scale volume electron microscopy (vEM). Lab-based X-ray facilities can be used for methods development to leverage proposals to use difficult-to-access synchrotron facilities and encourage wider application of X-ray imaging in all of life science. We welcome proof-of-concept studies and collaborations for interesting maize and adjacent research using X-ray imaging.

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

## P176

## Analysis of McClintock's tiny fragment/X component

(submitted by Taylor Isles <ti97d@umsystem.edu>)
Full Author List: Isles, Taylor D.[1]; Yang, Hua[1]; Albert, Patrice S.[1]; Gao, Zhi[1]; Birchler, James A.[1]
[1] Division of Biological Sciences, University of Missouri, Columba 65211

In 1978 McClintock described a fragment chromosome that could rearrange itself as well as become inserted into or attached to other chromosomes. McClintock attributed this to an "X component" on the fragment chromosome. She later produced a much-reduced version called "tiny fragment". This chromosome contained a centromere, near which she mapped the X component, attached to a short section of chromosome arm 9S. The marker genes *Shrunken1* and *Bronze1* could show evidence of loss, presumably by the failure of tiny fragment to be included in a developmental lineage. However, kernels were also observed that indicated a loss of function of either *Sh1* or *Bz1* alone. McClintock noted that many cases of *Bz1* mosaicism were typical of a silenced state with activation in small sectors in the endosperm and that these patterns could occur in clusters. We confirm all the observations of McClintock regarding kernel expression of *Sh1* and *Bz1* including evidence for some clusters exhibiting *bz1->Bz1* patterns. Derivatives have been recovered that have much higher meiotic transmission than the usual low frequency with some exhibiting Mendelian ratios as if tiny fragment were attached or inserted into another chromosome. One derivative shows a mosaic phenotype for *Bz1* through 15 generations of perpetuation. Whole chromosome paints show tiny fragment is composed solely of material from chromosome 9. It does contain a weak representation of CentC, suggesting that it has a native centromere. There are no detectable representations of 180 or TR1 knob repeats. Mapping read numbers along the length of the chromosomes from plants with tiny fragment compared to siblings without it suggests that tiny fragment contains 46 genes over approximately 2-3 Mb. Sequencing and whole chromosome paint technologies not available in McClintock's era should provide insight into the action of the "X component" to reorganize the genome.

## P177

## Capturing snapshots in history: Images from a collection of B. McClintock's microscope slides

(submitted by Paul Chomet <pchomet@gmail.com>)
Full Author List: Chomet, Paul[1]; Scroggs, Taylor[2]; Dawe, R. Kelly[2]
[1] Chomet Consulting LLC, Groton, CT, 06340
[2] University of Georgia, Athens, GA, 30606

During the summer of 1986, Dr. John Mottinger, University of Rhode Island, visited Dr. Barbara McClintock at Cold Spring Harbor Laboratory. To augment JM's cytogenetics class curriculum, Dr. McClintock donated 98 of her prepared microscope slides, along with notes and camera lucida drawings. Upon nearing retirement in 2014, Dr. Mottinger contacted PC to take possession of this slide collection. Recently, the authors captured digital images from the collection. The slides, produced by Dr. McClintock most likely in the 1930's and 1940's, are labeled with her handwriting and descriptions. In addition, a set of 6 slides prepared by Frances Janes Clark, who conducted research with Dr. McClintock during the summers of 1937-1939, was accompanied by a letter from FJC to BM dated Tuesday, April 30 (1940) verifying at least one date. The content of the collection contains a variety of maize chromosome spreads from plants containing translocations, ring and B chromosomes, and a maize-teosinte hybrid. The original plant material was labeled with McClintock, Rhoades, Stadler, Anderson and Burnham pedigree numbers. Also included is a set of fixed Neurospora spreads which undoubtedly came from McClintock's study of Neurospora during or soon after her visit to George Beadle at Stanford University (1944). With relevance to the discovery of transposable elements, slides containing mitotic spreads of leaf tissue show dicentric bridges demonstrating the "chromosome type" of the Breakage-Fusion-Bridge cycle. Additionally, one slide contains a meiotic pachytene image of a heterozygous chromosome 9 deletion; A chromosome configuration used in the induction of broken chromosomes. Controlling elements were discovered from plants that underwent the chromosome and chromatid type BFB cycles. This poster will place a few of these historical slide images (with accompanying notes) in context with McClintock's discoveries.

Funding acknowledgement: The Kelly Dawe homestead, Paul's wallet

# P178

## Chromosome axis provides scaffold for splicing meiotic gene transcripts

(submitted by Wojtek Pawlowski <wp45@cornell.edu>)
Full Author List: Du, Qian[1 2]; Wang, Minghui[1 3 4]; Tiang, Choon-Lin[1 5]; Sheehan, Moira J.[1 6]; Altendorf, Paul[7 8]; Yu, Ju-Kyung[7 9]; Hudecz, Otto[10]; Roitinger, Elisabeth[10]; Wang, Chung-Ju Rachel[11]; Meeley, Robert B.[12]; Ko, Clint[1 13]; Sun, Qi[2]; Golubovskaya, Inna[14]; Pawlowski, Wojtek P.[1]
[1] School of Integrative Plant Science, Cornell University, Ithaca, NY 14853, USA.
[2] Current address: Fujian Agriculture and Forestry University, Fuzhou, 350002, China.
[3] Bioinformatics Facility, Cornell University, Ithaca, NY 14853, USA.
[4] Current address: Meiogenix Inc., Ithaca, NY 14853, USA.
[5] Current address: Tunku Abdul Rahman University College, Kuala Lumpur, 50932, Malaysia.
[6] Current address: Breeding Insight, Cornell University, Ithaca, NY 14853, USA.
[7] Syngenta Biotechnology, Inc., Stanton, MN 55018, USA.
[8] Current address: CFH Ltd., Longmont, CO 80501, USA.
[9] Current address: Chungbuk National University, Cheongju, Chungbuk 28644, South Korea.
[10] Institute of Molecular Biotechnology, Vienna, 1030, Austria.
[11] Academia Sinica, Taipei, 11529, Taiwan.
[12] Pioneer Hi-Bred International, Inc. – A DuPont Company, Johnston, IA 50131, USA.
[13] Current address: The Rockefeller University, New York, NY 10065, USA.
[14] University of California, Berkeley, CA 94720, USA.

Progression though meiosis requires fine tuning of expression programs of many genes. This process includes promoting expression of genes required for meiosis and suppression of genes whose expression may interfere with meiosis. Although the core processes of meiosis exhibit strong evolutionary conservation in all eukaryotes, molecular mechanisms involved in meiosis transcriptome regulation vary between plants, animal, and fungi, and remain elusive. We discovered that the *Plural abnormalities of meiosis1* (*Pam1*) gene in maize facilitates a novel plant-specific type of meiosis progression control. *Pam1* encodes an RNA binding protein that directs meiosis transcriptome by associating with the meiosis-specific chromosome axis during early prophase I, binding transcript of a large number of meiosis-related genes, and affecting their splicing by interacting with the CCR4-NOT RNA processing protein complex. We hypothesize that the CCR4-NOT complex is present on meiotic chromosomes to facilitate co-transcriptional splicing and the role of the PAM1-mediated anchoring of CCR4-NOT to chromosome axis is to shield this process from the challenges of nuclear environment present in meiocytes but not in somatic cells. Disrupting *Pam1* function results in mis-spliced transcripts and causes several severe meiosis defects affecting chromosome condensation and behavior, as well as nuclear envelope and cytoskeleton organization. Interestingly, PAM1 regulates only a subset of meiotic genes and processes, suggesting that several programs directing transcriptome architecture collectively regulate meiosis progression.

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

## P179

### Copy number variation analysis of the cis region required for the B chromosome non-disjunction in maize

(submitted by Hua Yang <yanghu@missouri.edu>)
Full Author List: Yang, Hua[1]; Liu, Hua[3]; Liu, Jian[2]; Boadu, Frimpong[2]; Cheng, Jianlin[2]; Yang, Bing[3]; Birchler, James A.[1]
[1] Division of Biological Sciences, University of Missouri, Columbia, Missouri, USA 65211
[2] Department of Electrical Engineering and Computer Science, University of Missouri, Columbia, MO 65211
[3] Division of Plant Science & Technology, University of Missouri, Columbia, MO 65211

In maize, one of the components of the B chromosome drive mechanism is that it frequently undergoes nondisjunction at the second pollen mitosis, which produces one sperm with two copies of the B chromosome and the other sperm with no B. During this division, both a cis-acting region and trans-acting factors are required for the unequal allocation of the B chromosome. Previous genetic analyses showed that an F-box domain containing gene Zm00044a000666 ("666") is the trans-acting factor 1 required for nondisjunction. IP-MS showed that this gene interacts with SKP1 and RPA, suggesting that gene 666 is involved in a SCF E3 ligase complex and targets a single-strand DNA binding protein RPA at the second pollen mitosis. Previous analyses demonstrated that the cis region required for nondisjunction resides in the centromeric region of the B chromosome (Han et al., 2009). To address whether there is a difference of DNA replication in the centromere region between mutated (loss of nondisjunction due to mutant 666) and the normal B chromosome, we performed Copy Number Variation (CNV) analysis of three TB-9Sb mutant for nondisjunction versus the normal using mature pollen gDNA-seq data. The results showed that knob sequences in the centromeric heterochromatin region are at a lower copy number in normal B chromosomes than in the mutants. However, the B repeats in the centromere are at a higher copy number in the normal B chromosome compared to the mutants. Our results indicate that different replication outcomes of the knob and the B repeats around the B centromere region occur in the mutant and normal B chromosomes and might be involved in the mechanism of nondisjunction of the B chromosome at the second pollen mitosis.

Gene / Gene Models described: *666*; Zm00044a000666
Funding acknowledgement: National Science Foundation (NSF)

## P180 @Citogenetica

### Cytogenetic and genetic insights into non-mendelian segregation, non-disjunction, and phenotypic expression in the maize A-B chromosomal translocation line TB-9Sb

(submitted by Mateus Mondin <mmondin@usp.br>)
Full Author List: Said, Filipy Alves[1]; Oliveira, Eduardo Velloso[1]; Maronezi, Ana Laura Marassi[1]; Menuzzo-Molina, Silvia Cristina[1]; Aguiar-Perecin, Margarida Lopes Rodrigues[1]; Mondin, Mateus[1]
[1] Department of Genetics, "Luiz de Queiroz" College of Agriculture, University of São Paulo, Piracicaba-SP, Brazil, 13418-900

This study explores the cytogenetic and genetic mechanisms associated with the maize translocation TB-9Sb, emphasizing its effects on seed phenotypes and the behavior of atypical translocated A-B chromosomes. Fluorescence in situ hybridization (FISH) was used to analyze TB-9Sb and map the B-9 chromosomes, utilizing repetitive sequences such as K180 and B-specific markers. Two distinct B-9 chromosome variants were identified: one lacking the heterochromatic knob at K9S and carrying the C marker gene for colored aleurone (genotype TB-9Sb-2024a), and another possessing the knob at K9S and exhibiting a recessive phenotype for the C marker gene (genotype TB-9Sb-2024b). The study demonstrates that nondisjunction occurs in both aleurone tissue, albeit at a low frequency, and pollen grains in the TB-9Sb-2024a genotype. This research supports a non-Mendelian genetic model to explain nondisjunction in pollen grains and its impact on seed phenotypes. Additionally, a model for nondisjunction in aleurone tissue is proposed to explain the varying sectoring patterns, characterized by differences in expression intensity, observed in TB-9Sb-2024a seeds.Finally, karyotypes and insights into non-Mendelian genetic segregation and phenotypic expression in embryo and aleurone tissues are presented, offering a deeper understanding of the unconventional chromosomal dynamics associated with this translocation.

Funding acknowledgement: CNPq, PET-MEC

## P181 @Citogenetica

### Effects of B chromosomes on flowering time in Zapalote Chico inbred lines and the Cateto x Zapalote Chico hybrid

(submitted by Mateus Mondin <mmondin@usp.br>)
Full Author List: Ruas, Paulo Maurício[1 2]; Mondin, Mateus[1]; Garcia, Antonio Augusto Franco[1]; Maronezi, Ana Laura Marassi[1]; Aguiar-Perecin, Margarida Lopes Rodrigues[1]
[1] Department of Genetics, "Luiz de Queiroz" College of Agriculture, University of São Paulo, Piracicaba-SP, Brazil, 13418-900
[2] Department of General Biology, Center of Biological Science, State University of Londrina, Londrina-PR, Brazil, 86057-970

This study aimed to investigate whether the presence of B chromosomes influences the male and female flowering times in *Zea mays* L. To achieve this, four experiments were conducted, three using inbred lines of the Zapalote Chico variety and one with the hybrid Cateto x Zapalote Chico. The experiments were designed with different classes based on the number of B chromosomes, and a complete randomized block design was applied with two replications. The three experiments with Zapalote Chico inbred lines found that B chromosomes had minimal effect on female flowering time. However, the statistical analysis revealed that the male flowering time exhibited a significant variation, which could, at least in part, be attributed to the differences in the number of B chromosomes. In the Cateto x Zapalote Chico hybrid, the findings were consistent with those of the inbred lines, further supporting the observation that B chromosomes have a more pronounced effect on male flowering time. While the exact mechanism by which B chromosomes influence flowering time remains unclear, these results suggest that extranumerary chromosomes may alter flowering time, particularly in males. The potential roles of the euchromatic and heterochromatic regions of these B chromosomes in regulating flowering time are still unknown, and further research is necessary to elucidate the underlying mechanisms. This study contributes valuable insights into the genetic and phenotypic effects of B chromosomes in maize, highlighting the need for continued investigation to understand their role in flowering time regulation better.

Funding acknowledgement: CNPq, PET-MEC

## P182

### Genome-Wide investigation of recombination in the presence of B chromosomes

(submitted by Malika Sharma <msb92@umsystem.edu>)
Full Author List: Sharma, Malika[1]; Yang, Hua[1]; Liu, Jian[2]; Boadu, Frimpong[2]; Cheng, Jianlin[2]; Birchler, James A[1]
[1] Division of Biological Sciences, University of Missouri, Columbia, MO 65211
[2] Department of Electrical Engineering and Computer Science, University of Missouri, Columbia, MO 65211

B chromosomes (B) are supernumerary, dispensable chromosomes found in many species, including maize. Although not essential for development, B chromosomes have cytologically and genetically been shown to increase the rate of meiotic recombination, particularly in heterochromatic regions, indicating that they are not biologically inert. This study investigates the effect of B chromosomes on the maize genome across six BC1 populations. They are reciprocal crosses of (B73/W22 0B) X B73, (B73/W22 2-3B) X B73, and (B73/W22 6B) X B73. We aim to know whether the genome-wide crossover distributions are affected by the presence of B chromosomes, whether higher B chromosome number has a stronger effect than the lower B copy, whether B chromosomes affect interference, and whether this chromosome preferentially affects different regions of the genome such as heterochromatin. Through this analysis, we will identify genetic variants and chromosomal regions where crossover frequencies vary with the presence of B chromosomes. This work provides insight into the role of B chromosomes in influencing recombination and shaping the genomic landscape of maize.

Funding acknowledgement: National Science Foundation (NSF)

## P183

### Multiple trans-acting factors are required for B chromosome nondisjunction

(submitted by Nathan Gilbert <negvgc@umsystem.edu>)
Full Author List: Gilbert, Nathan E.[1]; Yang, Hua[1]; Birchler, James A.[1]
[1] Division of Biological Sciences, University of Missouri, Columbia, MO, USA 65211

In previous research, we discovered that a trans-acting factor located in the distal tip of the B chromosome called Gene 666 is necessary for nondisjunction of B chromosomes. However, it has not been established whether there are other trans-acting factors needed. In this research, we used a translocation, 9-Bic-1, which is a variant of chromosome 9 with an inactive B centromere located on the tip of the short arm and a transgene of gene 666 called 9-666, which is located in the long arm of chromosome 9. Previous work had shown that the inactive B centromere in 9-Bic-1 would attempt nondisjunction in the presence of B chromosomes, which could supply trans-acting factors. We then crossed 9-Bic-1 with 9-666 or 9-666 +2Bs mutant for 666 to determine whether 666 alone would restore nondisjunction. Our hypothesis was that there was no other trans-acting factor(s) required for the nondisjunction of our 9-Bic-1 x 9-666 F1. When we testcrossed with our F1 to a recessive on chromosome 9S (*c1*), the progeny showed a lack of nondisjunction. However, when the cross of 9-Bic-1, 9-666, and B's with a mutant 666 was used, evidence of nondisjunction or chromosome breakage of 9-Bic-1 was present. The chromosome breakage is caused by the nondisjunction of the inactive B centromere, while the separation of the centromeres of the 9-Bic-1 fractures one of the chromatids. These results indicate that gene 666 alone is necessary but not sufficient for nondisjunction and that when the remainder of the B chromosome was present, nondisjunction occurs. We conclude that other trans-acting factors beyond 666 are needed for the nondisjunction of the B chromosome. Funded by NSF MCB 2214243.

Funding acknowledgement: National Science Foundation (NSF)

## P184

### Reshaping the maize karyotype using synthetic centromeres

(submitted by Kelly Dawe <kdawe@uga.edu>)
Full Author List: Zeng, Yibing[1]; Wang, Mingyu[1]; Gent, Jonathan[1]; Dawe, Kelly[1]
[1] University of Georgia

Recent work has demonstrated the feasibility of engineering new centromeres by recruiting the key centromere protein CENP-A/CENH3 to defined locations. However, it has not yet been shown that engineered centromeres can accurately segregate chromosomes throughout all stages of an organismal life cycle, including meiosis. Here we describe a collection of maize neochromosomes (4b chromosomes) derived from sections of chromosome 4 containing engineered centromeres initially targeted to a synthetic repeat sequence. Analysis of the CENH3 profiles shows that CENH3 often spreads away from the synthetic repeat array and into flanking intergenic spaces. To demonstrate full functionality of one such synthetic centromere, we identified a truncated form of chromosome 4 (4a chromosome) and matched it with a complementing 4b chromosome. When both 4a and 4b were present in a homozygous state, the plants grew normally and displayed meiotic transmission that was indistinguishable from wild-type. The expression of 22 genes within the CENH3-occupied area of the 4b centromere were not substantially altered from the original wild type chromosome. Our data show that CENH3 tethering can result in fully functional centromeres, and that the method can be used to split an existing chromosome into two independently-segregating units.

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

## P185

### Single gene example of genomic balance using transcription factor *B-peru* on the B chromosome

(submitted by Hua Yang <yanghu@missouri.edu>)
Full Author List: Yang, Hua[1]; Singh, Jasnoor[1]; Sharma, Malika[1]; Liu, Jian[2]; Boadu, Frimpong[2]; Cheng, Jianlin[2]; Birchler, James A.[1]
[1] Division of Biological Sciences, University of Missouri, Columbia, Missouri, USA 65211
[2] Department of Electrical Engineering and Computer Science, University of Missouri, Columbia, MO 65211

Genomic balance refers to the fact that both increases and decreases of chromosomal dosage have a detrimental effect on plant stature and morphology. We have previously suggested that the balance phenomenon is a reflection of dosage sensitive regulatory genes. Our lab had recovered a transgene of the *B-Peru* transcription factor on the supernumerary B chromosome, which allowed us to produce an extensive dosage series to test the effect on its own expression as well as the target genes in the anthocyanin pathway. To pigment the plant, *B-Peru* and *Pl* are responsible for transcriptional activation and work together to regulate downstream genes *A1*, *A2*, *C2*, *Bz1*, *Bz2*, and *Pr*. In this study, we utilized W22 plants (null for *r1* and *b1*) with a *B-Peru* transgene inserted into a B chromosome (*B-PeruB*) and performed RNA-seq to examine the expression of *B-Peru* and the target anthocyanin genes. At low copy numbers of *B-Peru*, the targets increase in parallel with the dosage of *B-Peru*, reach a peak, and then decline even though the expression of *B-Peru* still increases. At very high copy numbers of the *B-PeruB*, *B-Peru* expression begins to decline as does the expression of the targets. These effects are also found on the phenotypic level. While the analysis was conducted on the RNA level and the transcription factors work on the protein level, the results suggest that this transcription factor has a peak effectiveness in relationship to its interactors establishing a single gene example of genomic balance.

Funding acknowledgement: National Science Foundation (NSF)

## P186

### Understanding the role of PRD2 in regulating meiotic recombination in maize

(submitted by Sora Haagensen <sorahaagensen@brandeis.edu>)
Full Author List: Haagensen, Sora[1 2]; Jing, Juli[1]; Pawlowski, Wojtek[1]
[1] School of Integrative Plant Science; Cornell University; Ithaca, NY USA 14853
[2] Department of Biology; Brandeis University; Waltham, MA USA 02453

Genetic variation is crucial for developing crops with higher yields, disease resistance, and other desirable traits. During plant meiosis, homologous recombination (HR) ensures this genetic variation by reshuffling genetic material between parental chromosomes. In Arabidopsis, the PRD2 gene initiates HR by regulating the formation of DNA double-strand breaks (DSBs). DSB ends are then resected to create overhangs that invade homologous DNA and form recombination intermediates, which are processed to form type I or II crossovers (COs). COs are formed through pairing and DNA synthesis between homologous chromosomes. Abnormal chromosome pairing can result in detrimental phenotypes such as sterility and aneuploidy. Pawlowski's group identified dsyCS, a mutant with a mutation on the Arabidopsis PRD2 homolog in maize, and showed a 98% decrease in DSBs compared to wild-type. Chromosome pairing and synapsis are defective in dsyCS, resulting in unequal chromosome segregation and the production of aborted gametes. Our experiments compared CO numbers between dsyCS and wild-type to uncover the function of the PRD2 gene in meiotic recombination and CO formation in maize.

Funding acknowledgement: National Science Foundation (NSF), Cornell University, Boyce Thompson Institute

## P187

### Utilization of engineered neochromosomes to study breakage-fusion-bridge cycle outcomes

(submitted by Kempton Bryan <kempton@uga.edu>)
Full Author List: Bryan, Kempton[1]; Dawe, Kelly R[1 2]
[1] Department of Genetics; University of Georgia, Athens, GA, 30602
[2] Department of Plant Biology; University of Georgia, Athens, GA, 30602

In our previous work, we have demonstrated the feasibility of centromere induction at a fixed location using a single transgene. In this system, a new centromere is formed on the long arm of chromosome 4, activating a breakage-fusion-bridge cycle (BFB) that liberates neochromosomes composed of fragments of the long arm with new centromeres. These neochromosomes provide a unique model to investigate stable BFB-derived haplotypes to better understand the way BFB can reorganize the genome. Here we present the structure of these neochromosomes. They show megabase scale structural variants including duplications, deletions, and inversions. De novo telomere addition has occurred, and stable telomere addition sites have been identified. Nanopore adaptive sampling will be used to study the structural variation on additional neochromosomes. This system also enables the study of the mechanisms of de novo telomere formation. High throughput assays will be used to find additional telomere addition sites. These data will be paired with cytological data to study the interaction between telomere formation and BFB cessation.

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

## P188

### Managing and distributing maize diversity: The NCRPIS maize collection

(submitted by Vivian Bernau <vivian.bernau@usda.gov>)
Full Author List: Bernau, Vivian M[1]; Millard, Mark J[1]
[1] 1. USDA/ARS Plant Introduction Research Unit and Iowa State University College of Agriculture and Life Sciences, Ames, Iowa

The USDA National Plant Germplasm System includes a collection of more than 20,000 accessions of cultivated temperate- and tropical-adapted maize and wild relatives from around the world. This collection is held at the North Central Regional Plant Introduction Station (NCRPIS) in Ames, Iowa. Currently, approximately 73% of the collection (14,941 accessions) is available for distribution upon request. Seed viability of each accession is monitored on a 5-10 year cycle. When viability drops below 50%, or if the number of kernels on hand falls below 1000, an accession becomes unavailable for distribution until it can be regenerated. Temperate-adapted material is typically regenerated in Ames, Iowa. In the past, nurseries have been provided by partners and contractors in the US and Mexico to regenerate diverse material from unique environments. Seed regeneration is costly and can negatively affect the genetic integrity of an accession. However, it is also an opportunity to gather further observations. GRIN-Global, the germplasm database of the NPGS, currently holds 376,312 trait observations on 17,478 accessions, and 48,097 ear, kernel, and cob images on 17,310 accessions. Germplasm requests can be made through the NPGS GRIN-Global public website. In 2024, more than 11,000 packets of maize were distributed by NCRPIS to requestors across the country and around the world.

Funding acknowledgement: United States Department of Agriculture (USDA)

## P189

## Reinventing tissue culture and transformation in the classroom: TPSS 499 - Track 2

(submitted by Jaclyn Nicole Uy <uyjnr69@hawaii.edu>)
Full Author List: Uy, Jaclyn Nicole[1]; Amore, Teresita[1]; Muszynski, Michael[1]
[1] Department of Tropical Plant and Soil Sciences, University of Hawai'i at Manoa, 3190 Maile Way, Honolulu, HI, 96822

Tissue culture is an essential skill for modern plant breeding techniques. TAE undergrad students have limited exposure to plant tissue culture: one lab session in TPSS 420, plant propagation and TPSS 440 Tissue Culture and Transformation. However, TPSS 440, "Tissue Culture/Transformation," is only offered alternate spring semesters, and has a strict minimum enrollment of 10 students. For students following a typical 4-year curriculum seeking training in plant tissue culture, we offered a semester long Undergraduate Directed Research course (TPSS 499) that introduced students to plant tissue culture and transformation techniques using the maize protocol developed by the GETSCI project At the beginning of the semester, students were required to attend a 3-hour modular lecture, with 1 hour focused on plant tissue culture fundamentals and 2 hours covering maize and bacterial transformation, followed by four weeks of intensive training on essential skills: media preparation, pipetting, aseptic techniques, and explant excision. For maize transformation students were trained to do embryo selection, transfection, regeneration and selection, and rooting. At the end of the semester, students are required to present their findings and reflections. This redesigned system has been tested over three semesters, with feedback collected from students at the end of each semester using an 8-question short-answer survey to assess the course's effectiveness and impact. To date, five students have participated in Track 2, all of whom provided positive feedback on the four-week mandatory training session. Students rated their confidence in independently performing transformations between 5.6 and 8 (on a scale of 1 to 10, with 10 being the highest). Many students have expressed a desire for more time to complete each project section. Given the semester’s timeframe, we are further modifying TPSS 499, which can already serve as an alternative to TPSS 420.

Funding acknowledgement: National Science Foundation (NSF)

## P190

## Teaching scientific writing alongside the scientific method in an introductory plant biology lab.

(submitted by Katy Guthrie <guthr103@umn.edu>)
Full Author List: Guthrie, Katy[1]; Levina, Anna[2]
[1] University of Minnesota; Saint Paul, MN, 55108
[2] Duke University; Durham, NC, 27708

Here, we describe an intentionally scaffolded writing curriculum in an introductory plant biology lab course, where students explored the scientific method while building scientific writing skills. The goal was to evaluate how early writing interventions impacted students' science writing ability. Our design is centered on the idea that writing instruction can be used to build scientific literacy (Yule et al., 2010), and that similar writing-interventions in introductory labs have had positive impacts on student preparedness (Dansereau et al., 2020). Students completed three research projects: The first introduced students to the scientific method through a structured lab where students tested a set hypothesis. In the second, students were provided with a research question, but created and tested their own hypothesis. The third project was inquiry-driven; students researched a topic of interest, created, then tested a hypothesis. All three projects explored core concepts in plant biology.Each project had an associated writing assignment, and instruction was scaffolded as follows: 1) data visualization and writing results, 2) writing a method and discussion section, 3) writing an introduction. This process was semi-iterative; skills taught in the first project were practiced and assessed in the second and third projects, and so on. Writing was evaluated through peer review via FeedbackFruits and final grades assigned by graduate teaching assistants (GTAs). Rubrics were intentionally designed to evaluate writing skill over content expertise. Preliminary results show that data visualization (figure development) saw marked improvement in 2022. However, in 2023, writing the results section proved to be a consistent, significant challenge for students, specifically their ability to describe the data in relation to the research question or hypothesis. This decrease in competency may be related to the increase in student-direction over lab projects, indicating a misalignment with identifying a research question, writing a hypothesis and subsequently selecting the correct variables to measure to address that hypothesis. These results indicate students may need more intentional scientific method instruction than previously thought.

Funding acknowledgement: Active Learning Initiative, Cornell University

## P191

## The MaGNET program: Fostering community and building networks through effective mentorship for early career scientists

(submitted by Brandi Sigmon <bsigmon2@unl.edu>)
Full Author List: Sigmon, Brandi[1]; Thompson, Beth[2]
[1] Department of Plant Pathology, University of Nebraska-Lincoln; Lincoln, NE, USA 68583-0722
[2] Department of Biology, East Carolina University; Greenville, NC, USA 27858-4353

The Maize Genetics Network Enhancement through Travel or MaGNET program aims to broaden participation in the maize genetics research community by decreasing barriers for scientists from underrepresented groups to attend the Annual Maize Genetics Conference. With a focus on first time attendees and early career scientists, the program provides full financial support (meeting registration and travel expenses) for approximately 8 scientists to attend the meeting each year. In addition to financial support, the MaGNET program supports awardees by providing mentoring and networking opportunities associated with the meeting. All MaGNET awardees are assigned to a mentoring group comprised of two awardees, an early career scientist (graduate student or postdoc), an industry or government scientist, and a senior academic researcher. These mixed mentoring groups provide opportunities for awardees to begin to build diverse professional networks while fostering community and a sense of belonging. Awardees also have the opportunity to present their research, including short lightning talks, learn of recent research advances in maize genetics and plant biology, and interact with invited speakers at MaGNET lunches. Through all these conference-related activities, the MaGNET program strives to promote STEM identity, belonging, and foster community for early career scientists through providing unique and impactful mentorship, networking, and professional development at the Maize Genetics Conference.

Funding acknowledgement: National Science Foundation (NSF)

## P192

## The organic corn breeding boot camp: An outreach activity for students, farmers and scientists

(submitted by Paul Scott <paul.scott@usda.gov>)
Full Author List: Scott, Paul[1]; Linares Ramirez, Angela[2]; Lübberstedt, Thomas[3]; Bohn, Martin[4]
[1] Corn Insects and Crop Genetics Research Unit, USDA-ARS, Ames, Iowa 500011
[2] Vegetable breeding and Crop Management Prod. Res. Program, University of Puerto Rico, Lajas, Puerto Rico 00667
[3] Department of Agronomy, Iowa State University, Ames, Iowa 50011
[4] Department of Crop Science, University of Illinois, Urbana, Il 61801

The Organic Corn Breeding Boot Camp is funded by a grant from the USDA- NIFA Organic Research and Education Initiative. The goal of the grant is to develop new maize varieties and breeding strategies to facilitate production of maize seed in organic systems. The boot camp has been held in each of the past three years in conjunction with our organic winter nursery pollination in Lajas Puerto Rico. Attendees include scientists (the PIs of the grant), farmers who produce organic corn and are interested in plant breeding and undergraduate students attending the University of Puerto Rico, Mayaguez. Travel costs are provided to allow attendees from the Midwest to travel to Puerto Rico in the winter and work and learn side by side with the student interns and the grant PIs in our winter nursery. In addition to the hands-on work experience, discussion sessions provide context that ties the field work to the larger objectives of the project and to the scientific theory behind the work. Discussion sessions also allow for the sharing of ideas between students, farmers and scientists. In the summer following the boot camp, student interns from Puerto Rico travel to the Midwest and assist with summer nurseries at the university locations. During this time, the student interns visit the farms of the boot camp farmers to learn about organic corn production in the Midwest. The boot camp provides a hands-on learning and information sharing opportunity for farmers, students and scientists interested in organic corn production.

Funding acknowledgement: United States Department of Agriculture (USDA)

# P193

## A Rootless1 knockdown allele affects maize nodal root development, increasing rooting depth, nitrogen uptake efficiency, and grain production in the field

(submitted by Alexander Liu <aliu@danforthcenter.org>)
Full Author List: Liu, Alexander E[1 2]; Thiruppathi, Dhineshkumar[2]; Bray, Adam L[2]; Bagnall, George[2]; Morales, Elisa[2]; Lebow, Clara[2]; Topp, Christopher N[2]
[1] Washington University in Saint Louis, Saint Louis, MO, USA 63141
[2] Donald Danforth Plant Science Center, Saint Louis, MO, USA 63132

Nodal roots dominate the maize root system and are critical for nutrient acquisition. Changing nodal rooting patterns could help improve root system function; for example, the "Steep, Cheap, and Deep" paradigm posits that high nodal root production just above the soil line, but no higher, could increase nitrogen capture. Rootless1, a classic maize mutant, produces very few nodal roots aboveground. We previously identified a large indel in the promoter of *ZmRt1* that reduces expression which we hypothesize is responsible for the original Rootless1 phenotype. However, an Ac/Ds insertion allele of *ZmRt1*, named *rt1-2*, was observed to induce supernumerary nodal roots near the soil line before a precipitous decline at higher nodes in several maize backgrounds. We present a multi-year and multi-location analysis of changes to root system architecture due to the *rt1-2* nodal rooting phenotype and functional effects on plant growth and nitrogen status. Nitrogen contrast field experiments were performed in 2022, 2023, and 2024 in Missouri and Colorado comparing the *rt1-2* allele to the *ZmRt1* wild type allele in conventional and nitrogen limited conditions. Root system architecture was analyzed from excavated root crowns, 1m soil cores, and minirhizotrons. X-ray tomography analysis of excavated root crowns showed significant differences in root crown development. Deep soil cores revealed that plants with the rt1-2 allele have increased root length deeper in the soil column while minirhizotron data collected over the growing season showed differences in root system growth over time. To measure the functional impact of the observed changes in root system architecture, aboveground measures including shoot biomass, shoot nitrogen, and grain production were collected. Plants with the *rt1-2* allele had higher concentrations of shoot nitrogen, potentially leading to the observed increase in grain in both conventional and low nitrogen conditions when compared to the *ZmRt1* wild type allele, suggesting a positive influence on root resource capture efficiency.

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

## P194

## A job for a cob? Enhancing cob sink strength as an approach for programming staygreen in maize

(submitted by Matthew Runyon <mrunyon2@illinois.edu>)
Full Author List: Runyon, Matthew J.[1]; Studer, Anthony J.[1]
[1] Department of Crop Sciences; University of Illinois Urbana-Champaign; Urbana, IL, USA 61801

Delayed maize senescence, which is controlled in part through optimizing the rate of carbon acquisition by source tissue with the rate of translocation and storage in sink tissues, is positively correlated with increased yield. Increasing the fraction of photoassimilate directed toward alternative sinks has the potential to prolong the duration of photosynthetic carbon assimilation, promoting a maximum realized grain yield. Previous data indicate that cob biomass traits such as diameter, mass, and density are positively associated with prolonged staygreen, suggesting relevance of this organ in regulating senescence as an alternative sink. To investigate this role, pollinated and nonpollinated $BC_2$ high-cob density introgressions in a non-staygreen and staygreen genetic background (B73 Hi-Cob and PHG35 Hi-Cob, respectively) were phenotyped for delayed senescence response and biomass partitioning compared to wild-type controls. On average, nonpollinated B73 Hi-Cob and PHG35 Hi-Cob plants reached full senescence 201 GDUs (29.5%) and 186 GDUs (17.4%) later than their nonpollinated wild-type B73 and PHG35 comparators, respectively. While pollinated plants of all genotypes accrued greater total biomass, biomass partitioning in alternative sinks was notably elevated in nonpollinated plants. Stalk and husk fractions were greater in nonpollinated plants of all genotypes, while primary cobs failed to grow as much as their pollinated counterparts. However, among nonpollinated plants, primary cobs of B73 Hi-Cob and PHG35 Hi-Cob were 101.8% and 82.4% heavier, and 19% and 16.6% more dense, than wild-type, respectively. While the correlation of cob density to photosynthetic potential appears dependent on the genetic backgrounds evaluated, standardized mid-cob diameter and mass measurements show a consistently positive correlation with late-season photosynthetic potential ($R$ = 0.765 and $R$ = 0.76). Collectively, these results support both a significant role of cob traits influencing the senescence rate of maize and the potential for selection of these traits to improve agronomic performance.

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

## P195

## A longitudinal study of the impacts of cover crop diversity on maize yield and nitrogen uptake

(submitted by Christopher Topp <ctopp@danforthcenter.org>)
Full Author List: Topp, Christopher N[1 2]; Bagnall, Cody[1 2]; Griffiths, Marcus[1 2]; Moran, Amelia[1 2]; Wong, Kong M[1 2]
[1] Donald Danforth Plant Science Center, St. Louis, MO, USA 63132
[2] Subteranean Influences on Nitrogen and Carbon Center of Excellence, St. Louis, MO, USA 63132

Cover cropping is considered a major conservation approach to improve ecosystem services for sustainable agriculture. However, clear barriers exist to widespread adoption of cover crops in maize agriculture. For example, a recent large-scale analysis using satellite and empirical data shows that cover crop adoption in the US Midwest, predominantly using cereal rye, can result in yield penalties of both maize and soybean. We contend that these apparent tradeoffs are not hardwired but rather due to major knowledge gaps in how diverse cover crops affect the soil and subsequently the cash crop. In this project, we are evaluating comprehensive root and shoot phenotypes of appx. 30 winter cover crop species and 10 mixtures and their effects on soil, and maize yield and nitrogen capture. The longitudinal experiment spanning 13 acres is in its third year and clear cover-crop genotype x maize yield interactions have been observed. In this poster we present the experimental design, cover crop phenotypic information, and maize yields and nitrogen content in Years 1 and 2. Ultimately, we seek to identify cover crops traits that enhance maize yield and nutrient capture to support breeding efforts that will incentivize cover crop adoption, thus increasing the sustainability of maize agriculture.

Funding acknowledgement: United States Department of Agriculture (USDA), Subterranean Influences on Nitrogen and Carbon Center of Excellence (Danforth Plant Science Center)

## P196 @zhongtaofor

### A mitogen-activated protein kinase kinase kinase gene conferring quantitative foliar disease resistance in maize

(submitted by Tao Zhong <tzhong3@ncsu.edu>)
Full Author List: Zhong, Tao[1]; Xue, Shang[2]; Olukolu, Bode[1]; Bian, Yang[2]; Wisser, Randall[3 4]; Holland, James[2 5 6]; Yang, Qin[7]; Balint-Kurti, Peter[1 5]
[1] Dept. of Entomology and Plant Pathology, North Carolina State University, Raleigh, NC, USA 27695
[2] Dept. of Crop and Soil Sciences, North Carolina State University, Raleigh, NC, USA 27695
[3] LEPSE, University of Montpellier, INRAE, Institut Agro Montpellier, Montpellier, FR 34000
[4] Dept. of Plant and Soil Sciences, University of Delaware, Newark, DE, USA 19716
[5] Plant Science Research Unit, USDA-ARS, Raleigh, NC, USA
[6] NC Plant Science Initiative, North Carolina State University, Raleigh, NC, USA
[7] State Key Laboratory of Crop Stress Resistance and High-Efficiency Production and College of Agronomy, Northwest A&F University, Yangling, China

Southern leaf blight (SLB), caused by the necrotrophic fungal pathogen *Cochliobolus heterostrophus*, is a major foliar disease of maize worldwide. Here, we used an association mapping panel consisting of 270 diverse inbred lines with high-density genetic markers to dissect the genetic basis of SLB resistance. Fourteen single nucleotide polymorphism (SNPs) variants were significantly associated with SLB resistance, thirteen of which co-localized with previously reported SLB quantitative resistance loci (QRL), corresponding to ten candidate genes. Using a combination of mutant and transgenic analysis we demonstrated that one of the candidate genes, *ZmMAPKKK45*, encoding a mitogen-activated protein kinase kinase kinase (MAPKKK), was the causal gene conferring SLB resistance at a locus defined by two associated SNPs on chromosome 3. We additionally demonstrated that *ZmMAPKKK45* conferred resistance to two other foliar disease of maize, northern leaf blight (NLB) and gray leaf spot (GLS), and enhanced production of reactive oxygen species (ROS) during the defense response. Furthermore, we found ZmMAPKKK45 enhances ROS production, probably by increasing the expression of maize respiratory burst oxidase homolog genes (*ZmRBOHs*) and we suggest that this is associated with its effect on multiple disease resistance.

Gene / Gene Models described: *mkkk45*; Zm00001d043900
Funding acknowledgement: National Science Foundation (NSF)

## P197

### A network-informed genomic prediction approach for plant architecture traits in maize and sorghum

(submitted by Edoardo Bertolini <EBertolini@danforthcenter.org>)
Full Author List: Bertolini, Edoardo[1]; Lipka, Alexander E.[2]; Eveland, Andrea L.[1]
[1] Donald Danforth Plant Science Center; St Louis, MO, USA 63132
[2] Department of Crop Sciences; University of Illinois at Urbana-Champaign; Urbana, IL, USA 61801

Plant architecture strongly influences planting density, which in turn drives productivity per unit area. Because architectural traits are often highly heritable, genomic prediction offers a powerful strategy to accelerate genetic improvement. Moreover, genomic prediction models can be used to probe the predictive ability of markers in specific genomic regions to explain the genetic architecture underlying traits.In this study, we leveraged developmental context-specific transcriptional networks that were generated to infer molecular processes occurring during maize tassel and leaf organogenesis, which underlie tassel branching and leaf angle traits, respectively. We tested whether genes identified through these gene regulatory networks contribute to the genetic architecture of important agronomic traits, tassel branch number and leaf angle, by assessing how markers in proximity to network-prioritized genes predict breeding values in two maize diversity panels. Predictive abilities of one reduced marker set associated with co-expressed network genes were comparable to those obtained when using the entire genome. However, a much smaller marker set based on proximity to highly connected transcription factors in core network motifs exhibited significantly higher predictive abilities than expected by chance in maize. Notably, we showed that this same core gene set could be used in translation to sorghum with equally high predictive abilities.Our findings suggest that functionally constrained transcription factors highly connected in network motifs facilitate the translation of biological information underlying plant architecture traits across diverse germplasm and closely related species. Such convergence of genetic regulators highlights promising avenues for targeted breeding aimed at optimizing architecture for high-density planting and improving agricultural productivity.
Funding acknowledgement: National Science Foundation (NSF), NSF-PGRP award #1733606

# P198

## Phenotypic evaluation of U.S. maize heirlooms in Columbia, MO

(submitted by Melissa Draves <madcfr@umsystem.edu>)
Full Author List: Draves, Melissa A.[1]; Cummings, Jordan M.[2]; Washburn, Jacob D.[3]; Gage, Joseph L.[2 4]; Holland, James[2 4 5]; Flint-Garcia, Sherry[3]
[1] University of Missouri; Division of Plant Science and Technology; Columbia, MO, USA 65211
[2] North Carolina State University; Department of Crop and Soil Sciences; Raleigh, NC, USA 27695
[3] USDA-ARS; Plant Genetics Research Unit; Columbia, MO, USA 65211
[4] NC Plant Sciences Initiative; Raleigh, NC, USA 27606
[5] USDA-ARS; Plant Science Research Unit; Raleigh, NC, USA 27695

Hybrid maize is the most productive crop in the United States, producing millions of bushels per year and is used for food, feed, and fuel production. Prior to maize hybrids, open-pollinated heirloom populations (landraces) were primarily used in the US for subsistence farming and livestock feed. Heirlooms are phenotypically and genotypically diverse, with many populations harboring unique alleles that are not observed in the modern germplasm pool due to the rapid selection for yield that drove hybrid production. However, heirlooms are relevant in specialty markets as many chefs, organic farmers, and smallholder growers are interested in marketing heirlooms as a natural culinary product. Outside of the United States, heirlooms have been intensively studied, with extensive monographs written about populations from Mexico, South America, and Europe. However, there are no comprehensive datasets on extant heirlooms from the United States. This study aims to fully phenotype 990 heirlooms, primarily housed at the North Central Regional Plant Introduction Station. Heirlooms were planted in a partially replicated design in the summer of 2024 in Columbia, MO and Clayton, NC. Manual phenotypic measurements including flowering time, leaf and tassel architecture, plant heights, ear disease, tillering data, and harvest traits were collected throughout the growing season. Unmanned aerial vehicles were flown each week to collect standardized images of the field, which will be used to extract whole plot traits including plant health and height. Preliminary analysis indicates that cluster analysis and summary statistics represent the variation between and within populations and provide insights into heirloom phenotypic relationships. Ongoing efforts include a repeated field trial in 2025, an automated ear and kernel imaging pipeline, and kernel composition analyses using near infrared spectroscopy. Ultimately, this project will produce a comprehensive, public data set that will fully describe heirloom populations and their potential use for novel culinary traits.

Funding acknowledgement: United States Department of Agriculture (USDA)

## P199

# Phenotypic evaluation and genotypic characterization of U.S. maize heirlooms in Clayton, NC

(submitted by Jordan Cummings <jcummin5@ncsu.edu>)
Full Author List: Cummings, Jordan M.[1]; Draves, Melissa A.[2]; Washburn, Jacob D.[3]; Gage, Joseph L.[1 5]; Holland, James[1 4 5]; Flint-Garcia, Sherry[3]
[1] North Carolina State University, Department of Crop and Soil Sciences, Raleigh NC 27695
[2] University of Missouri, Division of Plant Science and Technology, Columbia MO 65211
[3] USDA-ARS Plant Genetics Research Unit, Columbia MO 65211
[4] USDA-ARS Plant Science Research Unit, Raleigh NC 27695
[5] NC Plant Sciences Initiative, North Carolina State University, Raleigh NC 27606

Nearly all maize in the United States is grown through hybrid cultivation, but this hasn't always been the case. Before the first double-cross hybrid maize release in 1921, maize varieties were grown and maintained through open pollination and selective breeding. These open-pollinated (heirloom) varieties contain extensive phenotypic and genotypic diversity that has largely been closed off from modern breeding as it is currently poorly described. Heirloom maize is valuable in specialty markets, with many chefs, organic farmers, and growers interested in heirlooms as a natural culinary product. Heirlooms may also be a source of novel adaptive alleles and further elucidate the adaptive history of maize as it spread into the U.S. Outside the U.S., heirloom maize has been well characterized in monographs detailing the diversity of heirlooms developed in Mexico, South America, and Europe, but no such description of U.S. heirlooms exists yet. We examined the diversity of U.S. heirloom maize through a common garden experiment with a partially replicated augmented design of 990 heirloom populations, obtained from the North Central Regional Plant Introduction Station and seed-saver organizations, planted in Columbia, MO, and Clayton, NC. Phenotypic data for plant and ear height, flowering date, tassel and leaf architecture, tillering, lodging, and harvest traits were collected. Cluster analysis and summary statistics showed extensive phenotypic variation both within and between heirloom populations. In coming months, all 990 heirloom populations will be genotyped and field-based RNA samples will be sequenced for most. Genotyping, RNA sequencing, and an additional field season in 2025 will allow further characterization of the diversity present in U.S. heirloom maize. This project will produce a robust data set which will be publicly available to stakeholders for use in maize breeding.

Funding acknowledgement: United States Department of Agriculture (USDA)

# P200

## Analyzing the impact of breeding, planting density and nitrogen availability on maize root system architecture using X-ray imaging

(submitted by August Thies <athies@danforthcenter.org>)
Full Author List: Thies, August[1 2]; Sciarresi, Cintia[3]; Olmedo Pico, Lia B.[4]; King, Kyle A.[3]; Ruiz, Alejo[3]; Ferela, Antonella[3]; Ordóñez, Raziel A.[4]; Trifunovic, Slobodan[5]; Eudy, Douglas[5]; Vyn, Tony[4]; Archontoulis, Sotirios[3]; Topp, Christoper N.[1 2]
[1] Donald Danforth Plant Science Center; St. Louis, MO 63132
[2] University of Missouri; Columbia, MO 65211
[3] Iowa State University; Ames, IA 50011
[4] Purdue University; West Lafayette, IN 47907
[5] Bayer Crop Science; Chesterfield, MO, USA 63017

Root system architecture (RSA) is influenced by genetics, environments and agricultural management, thus understanding how these factors interact with each other is vital for root-based crop improvement. We examined the impact of maize breeding, environmental variation, plant density and nitrogen availability on RSA through comparisons of root traits from a panel of era hybrids. To observe planting density, twelve maize hybrids were selected from across four decades (1980s-2010s) of the Bayer Crop Science breeding program and planted at both the historical density of the hybrid and a modern density at three different fields in Iowa, Indiana, and Illinois in 2021 and 2022. We also planted these maize hybrids and an additional fifteen at a control fertilizer rate and a low rate at three similar field sites. To explore differences in RSA, we shovel-excavated over 2000 root crowns at stage R3. These samples were scanned using X-ray computed tomography and we created digital 3D reconstructions using a custom computational pipeline. From these reconstructions, we calculated more than 100 root traits. Additionally, we measured the local climates, soil nitrogen, bulk density and water table depth to better understand which environmental parameters were correlated with any changes in RSA. We found that breeding, environmental variation and planting density all account for root crown phenotypic variation and there is a change in modern maize RSA. Differences in modern maize root crown RSA suggested, when planted at higher planting densities, modern maize lines have an improved capacity to maintain space, further root exploration and greater root intercolation with neighboring plants. These findings will improve our understanding of the heritability of root traits and the underlying genetics that control RSA development across a major US breeding program, which can help improve future maize hybrids.

Funding acknowledgement: Foundation for Food & Agriculture Research (FFAR)

## P201

# As above leaf below: integrating drone imagery and leaf gas exchange measurements from the Wisconsin Diversity Panel in different Nitrogen treatments

(submitted by Brandon Webster <webst250@msu.edu>)
Full Author List: Thompson, Addie[1]; Webster, Brandon[1]
[1] Michigan State University, East Lansing, MI, USA 48804

Plant breeding has continuously improved gains in Nitrogen efficiency by raising the yield ceiling in response to more intense fertilization. A major component of response is extended metabolic activity after silking, termed stay-green because of the longer retention of photosynthetic pigments. The color of the canopy can be a conspicuous indicator of general metabolic in the lead up to senescence. However, it remains unclear what exactly this means in terms of the flux of water and carbon molecules through leaf stomata. Further, "green" is a subjective metric. To quantitatively understand how metabolic components of stay-green affect yield in maize, the Wisconsin Diversity Panel was grown in contrasting N-fertilizer treatments (low/high). Measurements of gas exchange parameters A, E, Ci - $CO_2$ assimilation rate, $H_2O$ transpiration, and intercellular $CO_2$ concentration respectively, were made in two years from a subset of the panel approximately 20 days after average silking date. Simultaneously, ortho-imagery was collected via drone to assess how quantitative measures of spectral properties associate with gas exchange. Preliminary results reveal that N-fertilizer status can make a big impact on the relationship between yield and continued gas exchange after flowering. High and low input plots differed in terms of A only ($p=0.03$). In low N, both A ($r = 0.25$) and E ($r = 0.26$) were significantly positively associated with yield. In high N, the importance of A decreased ($r = 0.15$) while E remained significant ($r = 0.3$). Similarly, plot level vegetative indices were strongly associated with both A and E in low N, but not in high N. Together these results help show how N-context affects components of stay-green and the ability to detect those differences from aerial imagery. Further investigations aim to leverage genetic markers in the Wisconsin Diversity panel to investigate genetic variation that exists for these traits.

Funding acknowledgement: National Science Foundation (NSF)

# P202

## BZea: A diverse teosinte introgression population for improving modern maize sustainability

(submitted by Hannah Pil <hdpil@ncsu.edu>)
Full Author List: Pil, Hannah D[1 2]; Insko, Lauren[1]; Glover, Zehta S[1 2]; Tandukar, Nirwan[1 2]; Escalona Weldt, Carolina[1]; Stokes, Ruthie[1]; Rodríguez Zapata, Fausto[1]; Fritz, Katelyn[3]; Romay, Cinta[4 5]; Barnes, Allison C[1 6]; Gage, Joseph L[3]; Buckler, Edward S[4 5 7]; Sawers, Ruairidh[8]; Holland, James B[3 6]; de Jesús Sánchez González, José[9]; Rellán Álvarez, Rubén[1]
[1] Department of Molecular and Structural Biochemistry, North Carolina State University, Raleigh, NC, USA.
[2] Genetics and Genomics, North Carolina State University, Raleigh, NC, USA.
[3] Department of Crop and Soil Sciences, North Carolina State University, Raleigh, NC, USA.
[4] Institute for Genomic Diversity, Cornell University, Ithaca, NY, USA.
[5] Section of Plant Breeding and Genetics, Cornell University, Ithaca, NY USA.
[6] USDA-ARS, Plant Science Research Unit, Raleigh, NC, USA.
[7] USDA-ARS, Robert W. Holley Center for Agriculture and Health, Ithaca, NY, USA.
[8] Department of Plant Science, Pennsylvania State University, State College, PA, USA.
[9] Universidad de Guadalajara, Centro Universitario de Ciencias Biológicas y Agropecuarias, Zapopan, Jalisco, Mexico.

Teosinte, the wild ancestor of maize, harbors a rich reservoir of interesting traits that have been largely diminished during maize domestication. Recognizing the potential of these unknown alleles, here, we present the genotypic and phenotypic characterization of a new teosinte introgression population and preliminary data on experimental applications of this germplasm. Its donor lines consist of 81 georeferenced teosinte accessions from different species of the Zea genus, including Zea mays ssp. parviglumis, ssp. huehuetenangensis, ssp. mexicana, Zea diploperennis, and Zea luxurians, with around 2100 total derived lines. Evaluating the impact of teosinte alleles on agronomic traits is inherently difficult due to the differences in photoperiod and growth habitat of maize. This population, "BZea," addresses this challenge by creating BC2S3s with B73, creating derived lines with 12.5% of the original teosinte donors. Introgression into B73 allows for the evaluation of teosinte alleles in a maize background in temperate conditions. To characterize this population, we conducted whole genome sequencing of 1400 of these derived lines with an average sequencing depth of 0.8x. We have also performed 3' RNA-sequencing on a subset of this population. Using this population, we are investigating diverse experimental questions. We are currently exploring nitrogen dynamics by assessing lines derived from Z. diploperennis, a perennial teosinte that we expect to harbor alleles relevant to nutrient recycling. Furthermore, we are also using this population to generate allelic series for several candidate genes, including those relevant to nitrogen and other abiotic factors. These efforts aim to uncover the functional impact of teosinte alleles and their potential to enhance maize agronomic traits.

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

## P203

## Breeding for high lysine content and color diversity in popcorn and sweet corn to enhance their macro- and micronutrient profiles

(submitted by Jonathan Niyorukundo <niyorukundoj@gmail.com>)
Full Author List: Niyorukundo, Jonathan[1]; Yobi, Abou[2]; Kramer, Lucas[3]; Anderson, William[1]; Angelovici, Ruth[2]; Holding, David[1]
[1] University of Nebraska-Lincoln; 1400 R St; Lincoln, NE, 68588
[2] University of Missouri-Columbia; Columbia, MO, 65211
[3] Grand View University; 1200 Grandview Ave; Des Moines, IA, 50316

Zein proteins dominate maize endosperm protein content but lack essential amino acids like lysine and tryptophan. The opaque-2 (o2) transcription factor mutation decreases alpha-zeins and increases non-zeins through proteome rebalancing, enhancing protein-bound lysine. Building on insights from the Quality Protein Popcorn (QPP) project, this study aims to develop high-lysine popcorn and sweet corn varieties. Colored popcorn and sweet corn varieties (sugary-1 and shrunken-2) were crossed with quality protein maize to create high-lysine variants. Popcorn lines underwent selfing and backcrossing to retain the optimal popcorn background while selecting vitreous kernel lines with the gamma-zein o2 modifier gene. The project is ongoing, aiming to produce high-lysine, colored popcorn inbreds In the sweet corn project, F2 kernels segregating for the *o2* mutation and sweet corn traits advanced to F5 and were selected for homozygous o2 mutation, sweetness, and texture. Resultant lines exhibited increased lysine levels, with some surpassing their sweet corn parents. These sweet corn varieties were also crossed with color donors (popcorn, dent, and flint corn) and advanced to F5 through multiple selections for color, sweetness, and texture at 20 days post-pollination. Sugar testing for sucrose, glucose, and starch content was conducted Colored sweet corn was also analyzed for flavonoid and carotenoid pigments. These colored varieties maintained sugar content similar to their sweet corn parents with minimal variation in sweetness and texture. Additionally, some lines showed increased and diverse levels of flavonoids and carotenoids, suggesting added nutritional benefits associated with color pigments.

## P204

## Breeding for reduced maize grain nitrogen content

(submitted by Anthony Assibi Mahama <maassibi@gmail.com>)
Full Author List: Mahama, Anthony A[1]; Scott, Paul M[2]; Flint-Garcia, Sherry A[3]; Hirsch, Candice N[4]
[1] ORAU-ORISE/REE-ARS, 2104 Agronomy Hall, 716 Farm House Ln, Iowa State University Ames, IA 50011
[2] USDA-REE-ARS, 1407 Agronomy Hall, 716 Farm House Ln, Iowa State University, Ames, IA 50011
[3] Department of Agronomy and Plant Genetics, University of Minnesota, 199 Upper Buford Circle, St Paul, MN 55108
[4] USDA-ARS 301 Curtis Hall, University of Missouri, Columbia, MO 65211

Protein is an important component of maize grain that is used for food or feed. It is less important for other uses such as biofuel production. Protein production requires high levels of nitrogen fertilizer which reduces water quality and contributes to global warming, creating an incentive for low protein corn that requires less nitrogen fertilizer and can be used for applications that do not require high levels of protein. One of the objectives of the Circular Economy that Reimagines Corn Agriculture (CERCA) project is to develop corn populations with low grain nitrogen through conventional breeding, i.e., recurrent selection. We carried out two cycles of selection for low grain protein concentration in three different populations. The selection was replicated at locations in MN, IA and MO. NIR spectroscopy was used to predict protein content (which can be translated to nitrogen content using an established conversion factor) and make selections. In addition, the content of other grain components was predicted as well. In this poster, we present results comparing mean protein content of selected and unselected control populations to ascertain any progress made towards our goal of developing corn populations with low grain protein content

Funding acknowledgement: United States Department of Agriculture (USDA)

# P205
## Breeding maize stover for a circular bioeconomy
(submitted by Samuel Herr <skh77@cornell.edu>)
Full Author List: Herr, Sam[1]; Wickes-Do, Liam[1]; Farmer, Erin[1]; Jusino Montalvo, Ela[2 3]; Yan, Ruyu[4]; Michael, Peter[4]; Gu, Zeqi[5]; Daza Castañón, Amparo S.[6]; Lepak, Nicholas[7]; Harman-Ware, Anne E.[8]; de Leon, Natalia[6]; Okumoto, Sakiko[9]; Ainsworth, Elizabeth A.[10]; Davis, Abe[4]; Romay, M. Cinta[11]; Buckler, Edward S.[1 7 11]; Emmett, Bryan[2]; Gore, Michael A.[1]
[1] School of Integrative Plant Science, Plant Breeding and Genetics Section, Cornell University, Ithaca, NY 14853, USA
[2] USDA-ARS National Laboratory for Agriculture and the Environment, Ames, IA, 50011, USA
[3] Oak Ridge Institute for Science and Education, US Department of Energy, Oak Ridge, TN
[4] Department of Computer Science, Cornell University, Ithaca, New York 14853, USA
[5] Cornell Tech, New York, New York 10044, USA
[6] Department of Plant and Agroecosystem Sciences, University of Wisconsin—Madison, Madison, WI 53706, USA
[7] USDA-ARS Robert W. Holley Center for Agriculture and Health, Ithaca, NY 14853, USA
[8] Renewable Resources and Enabling Sciences Center, National Renewable Energy Laboratory, Golden, CO, 80401, USA
[9] Department of Soil and Crop Sciences, College of Agriculture and Life Sciences, Texas A&M University, College Station, 77843, TX, USA
[10] Department of Plant Biology, University of Illinois Urbana Champaign, Urbana, IL 61801, USA
[11] USA Institute for Genomic Diversity, Cornell University, Ithaca, NY 14853, USA

Maize stover, the aboveground biomass left after grain harvest, is an abundant carbon resource that could play a vital role in bioeconomies due to its use for livestock, soil management, and bio-based products. Lignin, a main component of stover, is a complex aromatic polymer essential for plant growth and abiotic/biotic stress resistance. However, because of its recalcitrant nature, the concentration and composition of lignin can affect the value of stover for use in chemical and feed industries. In addition, lignin impacts the decomposition rate of stover in soil, influencing its role in sustainable maize agroecosystems. While breeding stover for a favorable lignin composition is vital for economic use, it is unknown how these changes in composition affect soil health. We explored two maize stover research areas: 1) genomic and phenomic prediction of maize stover lignin composition, and 2) the effect of lignin composition on soil carbon and nitrogen cycles during stover decomposition. For our first objective, we performed pyrolysis molecular beam mass spectrometry to quantify the relative lignin composition and concentration in stalk tissue from a Genomes To Fields population of ~500 maize hybrids. In addition, we collected multi-spectral imagery and lidar on these maize hybrids over the growing season. Using data from the first year, we achieved moderately high genomic and phenomic prediction accuracies for lignin composition and concentration. To investigate how variation in stover lignin composition impacts its decomposition and, subsequently, soil carbon and nitrogen cycling, we used *brown midrib* mutants--naturally occurring mutants with extremely low lignin concentrations--in a litterbag experiment. Preliminary results show non-significant effects of lignin composition on nitrogen dynamics, including inorganic nitrogen, nitrous oxide emissions, and potential nitrification rate. Future research will focus on understanding how different environments influence lignin composition and how changes in lignin composition affect the soil microbiome during stover decomposition.

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## P206
## CERCA - Circular Economy that Reimagines Corn Agriculture

(submitted by Cinta Romay <mcr72@cornell.edu>)
Full Author List: Romay, M. Cinta[1]; Barnes, Allison C[2]; Ojeda, Jonathan O[1]; team, CERCA[5]; Buckler, Edward S.[1 3 4]
[1] Institute for Genomic Diversity, Cornell University, Ithaca, NY 14853, USA
[2] USDA-ARS, Raleigh, NC, 27695, USA
[3] School of Integrative Plant Sciences, Plant Breeding and Genetics Section, Cornell University, Ithaca, New York, 14853, USA
[4] USDA-ARS, Ithaca, NY 14853, USA
[5] US labs across the country

Maize stands as the world's most productive grain crop and a cornerstone of the global food supply. In industrial agriculture, it is primarily valued for its efficiency in starch production, with its protein content having relatively modest value. However, protein is the key driver behind most of the fertilizer demand, which constitutes the largest energy input for maize cultivation. The current combination of genetics and agronomic practices for high-yielding industrial corn leads to elevated costs for farmers, water pollution, and greenhouse gas emissions through the release of nitrous oxide. This poster highlights the progress of CERCA (Circular Economy that Reimagines Corn Agriculture), a project dedicated to enhancing maize's photosynthetic and nitrogen efficiency while adapting the crop for modern applications and annual farm rotations. A team of over 80 scientists is engaged in three key areas of research: (1) discovery and engineering of genetics and physiologies for cold germination and growth observed in related species to better adapt maize to the wet and cold conditions of early spring, when natural nitrogen is abundant (2) eliminate poor-quality storage proteins and develop perennial-like traits for late-season photosynthesis and efficient remobilization of nitrogen and phosphorus to roots, cobs or other storage organs to reduce maize's nitrogen demand (3) biological nitrification inhibition from residue and exudates to stabilize soil nitrogen and integrate better with a suitable cropping system. The outcomes of this research endeavor would be nitrogen-efficient grain production that enhanced farmer planting flexibility, reduced fertilizer inputs, and reduced environmental impact.

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

## P207 @burnsmj7
## CHIP-NMC: an application for corn hybrid and inbred prediction of nixtamalization moisture content

(submitted by Michael Burns <burns756@umn.edu>)
Full Author List: Burns, Michael J.[1]; Berry, Sydney P.[1]; Loftus, Molly[1]; Gilbert, Amanda M.[1]; Hirsch, Candice N.[1]
[1] Department of Agronomy and Plant Genetics, University of Minnesota, St. Paul, MN 55108

The quantity of water absorbed during the nixtamalization of maize greatly influences the final product's taste, nutritional profile, and machinability. A machine learning model that uses near-infrared spectroscopy to predict the moisture content of nixtamalized maize inbred lines was previously developed. Inbred and hybrid maize differ in many ways including shape, size, and composition of kernels, which can all affect nixtamalization moisture content. The inbred model was assessed for application with hybrid germplasm, the primary input for most industrial uses, and a low Spearman correlation coefficient of 0.539 was observed. A new model trained on diverse hybrid maize was developed and validated. The hybrid model achieved a Spearman's rank correlation coefficient of 0.815 across five populations of food-grade and non-food-grade maize. The hybrid model was used to assess relationships between grain compositional properties and nixtamalization moisture content and significant relationships with fat and fiber content were found. The hybrid model developed here and the previous inbred model have been incorporated into a Shiny R application called CHIP-NMC, which can be incorporated into various stages in the masa-based product development chain including breeding, elevator acceptance, and manufacturing.

Funding acknowledgement: PepsiCo Inc., University of Minnesota Informatics Institute, University of Minnesota Graduate School

## P208

## Characterizing tissue-specific disease resistance to *Xanthomonas vasicola* pv. *vasculorum* in maize

(submitted by Alexander Mullens <mullens3@illinois.edu>)
Full Author List: Mullens, Alexander[1]; Rapciak, Robert[1]; Wang, Shuya[1]; Zhong, Tao[2]; Balint-Kurti, Peter[2 3]; Jamann, Tiffany[1]
[1] Department of Crop Sciences, University of Illinois, Urbana, Illinois, 61801
[2] Department of Entomology and Plant Pathology, North Carolina State University, Raleigh, North Carolina 27695
[3] Plant Science Research Unit, U.S. Department of Agriculture-Agricultural Research Service, Raleigh, North Carolina 27695

The xylem and mesophyll represent distinct habitats within a plant for pathogenic bacteria to colonize. Host plants often utilize different mechanisms to defend themselves against xylem colonizing and mesophyll colonizing pathogens. *Xanthomonas vasicola* pv. *vasculorum* (*Xvv*) is an emerging bacterial pathogen of maize. It is commonly described as a foliar mesophyll colonizing pathogen in maize, but as a xylem colonizing pathogen in sugarcane. However, rare examples of xylem colonization in maize have also been reported. The maize *Xvv* pathosystem offers a unique opportunity to study tissue-specific host resistance in response to xylem and mesophyll colonization exhibited by a single phytopathogenic bacteria. Here we report the use of differential inoculation techniques, florescence microscopy, RNA-Seq and linkage mapping to show that (i) different inoculation techniques can be used to induce xylem or mesophyll colonization; (ii) genetic resistance to *Xvv* in the xylem and mesophyll are under independent control; (iii) there are striking differences in expression of genes relating to motility and virulence in *Xvv* when it is inhabiting the xylem versus the mesophyll; and (iv) there are significant expression differences for genes relating to plant defense in resistant and susceptible plants within the QTL intervals that we mapped; (v) UniformMu lines with insertions in candidate resistance genes are more susceptible to *Xvv*. This research is significant because it contributes to the limited knowledge regarding the genetics of resistance to *Xvv* in maize and offers insights into how *Xvv* adapts to the xylem and mesophyll.

Funding acknowledgement: National Science Foundation (NSF)

## P209

## Characterizing untapped genetic diversity in maize and its relatives to regulate the rhizosphere microbiome and improve crop resilience

(submitted by Nate Korth <njkorth@ncsu.edu>)
Full Author List: Korth, Nate[1]; Fritz, Katelyn[1]; Gage, Joseph L[1]
[1] North Carolina State University, Raleigh, NC, 27606

To advance crop improvement in the face of climate change, it is critical to characterize and harness previously untargeted traits, such as the composition and function of the plant-associated microbiome. Shifting microbial communities associated with plants offers a promising strategy to enhance crop resilience by improving nutrient cycling and bolstering resistance to abiotic and biotic stress. However, mechanisms for microbiome regulation may have been unintentionally lost during modern breeding and domestication. Leveraging genetic diversity from related species, such as teosinte and sorghum, which exhibit tolerance to abiotic stressors that maize is sensitive to, offers a path to recover these mechanisms. Studying these relatives alongside maize provides an opportunity to characterize the genetic architecture underlying microbiome modulation for improved resilience. Environmental conditions further influence the phenotypes of maize and its microbiome, with differential effects depending on the plant genotype and microbial composition. We propose that the plant-associated microbiome underpins some mechanisms of genotype-by-environment (GxE) interactions. To investigate this hypothesis, we conducted an experiment to measure the functional characteristics of maize, sorghum, and teosinte roots and their associated microbiomes under heat stress using a dual RNAseq approach. By integrating microbiome measurements into a GxE framework, we found associations between maize gene expression and microbial pathway enrichment, providing insights into how the plant microbiome contributes to heat resistance. This research contributes to the foundation for utilizing plants' ability to alter their microbiomes to enhance their resilience to environmental stressors and improve adaptability in a changing climate.

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

## P210 @AmanArora_7

### Cis-acting natural variation in transcript abundance affects the phenotype of the semi-dominant *D13-1* mutant

(submitted by Amanpreet Kaur <kaur60@purdue.edu>)
Full Author List: Kaur, Amanpreet[1 2]; Khangura, Rajdeep S.[1 2]; Dilkes, Brian[1 2]
[1] Department of Biochemistry, Purdue University, West Lafayette, Indiana, USA 47907
[2] Center for Plant Biology, Purdue University, West Lafayette, Indiana, USA 47907

Dwarf mutants are valuable resources for studying the genetic mechanisms that regulate plant height and architecture. They serve as links between genotype and phenotype, allowing the examination of genetic variations and their effects on the phenotype. The interactions of the mutant gene with other regions in the genome can be studied by mapping the modifiers that alter the mutant phenotype. Dominant and semi-dominant mutants can be crossed to diverse maize lines to generate F1 association mapping populations (FOAM), enabling the identification of loci that enhance or suppress the mutant phenotypes and the assignment of natural variants to molecular pathways. Unlike most QTL and GWAS approaches, when the identity of the mutant gene is known, the discovery of natural variants with phenotypic impacts as epistatic modifiers of mutant alleles assigns these variants to the mutant pathway. GWAS in a FOAM population allows for high-resolution mapping of genetic modifiers that affect the mutant phenotype. We utilized this approach with the *D13-1* mutant, a semi-dominant dwarf mutant of maize carrying a point mutation in a glutamate receptor-like ion channel. By crossing *D13-1/+* to hundreds of maize inbreds we identified natural variation modifying the *D13-1/+* phenotype. Each F1 family segregated 1:1 for wild type and *D13-1* plants, F1 hybrid siblings that differed only at the *d13* locus providing matched congenic controls for every mutant. This identified natural variation at *d13*, as well as several other glutamate receptors, and genes encoding proteins that interact with glutamate receptors, affecting the severity of the *D13-1/+* phenotype. By integrating these data with expression level GWAS, we identified alleles affecting *d13* expression and demonstrated that a strong cis-acting regulatory variation affecting the transcript abundance of *d13* can suppress the *D13-1* phenotype.

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

## P211

### Combining ability and heterotic responses among newly developed elite stay-green sorghum inbred lines

(submitted by Nathan Bowser <nbowser@iastate.edu>)
Full Author List: Bowser, Nathan[1]; Ejeta, Gebisa[2]
[1] Iowa State University; Ames, IA, 50014
[2] Purdue University; West Lafayette, IN 47905

Sorghum [Sorghum bicolor (L) Moench], a crop grown in arid and semi-arid environments, can be enhanced with the stay-green trait to improve post-flowering drought tolerance. Previous studies on stay-green sorghum hybrids have noted a positive association between stay-green and grain yield in low potential environments, but did not assess the combining ability or heterotic responses of individual genotypes. In this study, stay-green seed parents developed at Purdue University were crossed with three elite pollinators, and their combining ability and heterotic responses were assessed in multiple locations which represented both stress and non-stress environments. Results showed greater variation in general and specific combining ability estimates in stress environments compared to non-stress environments, with a higher proportion of additive genetic variance compared to non-additive variance in both types of environments. Levels of heterosis for grain yield, 1000 seed weight, and plant height varied across locations and between R-lines. Several experimental hybrids derived from the stay-green inbred lines outyielded commercial hybrid checks in the Ethiopian environments while showing good hybrid seed production potential, with high seed parent yield and good nicking.

Funding acknowledgement: Bill & Melinda Gates Foundation

## P212

## Complex traits: Genetic diversity, environmental context, and phenotypic plasticity

(submitted by Xianran Li <xianran.li@usda.gov>)
Full Author List: Li, Xianran[1]; Guo, Tingting[2]; Mu, Qi[3]; Tibbs-Cortes, Laura[4]; Han, Linqian[5]; Vanous, Adam[6]; Yu, Jianming[7]
[1] USDA-ARS, Wheat Health, Genetics, and Quality Research Unit, Pullman, WA, USA 99164
[2] National Key Laboratory of Crop Genetic Improvement, Huazhong Agricultural University, Wuhan, China 430070
[3] Department of Plant and Soil Sciences, University of Delaware, Newark, DE, USA 19716
[4] USDA-ARS, Corn Insects and Crop Genetics Research Unit, Ames, IA, USA 50014
[5] Department of Crop and Soil Sciences, Washington State University, Pullman, WA, USA 99164
[6] USDA-ARS, North Central Regional Plant Introduction Station, Ames, IA, USA 50014
[7] Department of Agronomy, Iowa State University, Ames, IA, USA 50011

Complex trait dissection and prediction have been two major research areas. Understanding the genetic and environmental factors behind phenotypic variation can help answer some longstanding biology questions and improve phenotype prediction. While genetic diversity has been studied extensively, attention to phenotypic plasticity, the property of a genotype to produce different phenotypes under different environmental conditions, has been limited. Through a set of focused studies of multiple traits in multiple crops (maize, sorghum, rice, wheat, and oat), we have recently developed an integrated framework for gene discovery underlying phenotypic plasticity and performance prediction across environments. With an identified environmental index to quantitatively connect environments, a systematic genome-wide performance prediction approach was established through either genotype-specific reaction norm parameters or genome-wide marker-effect continua. These parallel genome-wide approaches were demonstrated for in-season and on-target performance prediction by simultaneously exploiting genomics, environment profiling, and performance information. At the same time, the effects of genes, QTLs, and GWAS peaks were visualized along the environmental index to highlight the environmental context of genetic effects, i.e., the gene-environment interplay. With additional multi-stage measurements of plant height, genetic effect continua both along the environmental gradient and along the developmental stage were revealed with the 3-D reaction norms. A conceptual model was proposed to showcase the interconnected components underlying the observed phenotypic variation: genotype, environment, and development, and at the three levels: single locus, multi-locus haplotype, and individual organism. By introducing the evolutionary-time dimension, we evaluated the patterns of phenotypic plasticity change during crop improvement. This general framework and the companion CERIS-JGRA analytical package should facilitate biologically informed dissection of complex traits, enhanced performance prediction in breeding for future climates, and coordinated efforts to enrich our understanding of mechanisms underlying phenotypic variation. We propose to further integrate development and physiology at the whole-plant level and gene expression network at the molecular level to enhance our understanding of the mechanisms of how phenotypes of complex trait emerge.

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

## P213

## Comprehensive kernel analysis in large DH populations derived from intercrossing germplasm enhancement of maize lines

(submitted by Kiara Kappelmann <kiarak@iastate.edu>)
Full Author List: Kappelmann, Kiara[1]; Li, Xianran[2 3]; Yu, Jianming[1]; Vanous, Adam[1 4]
[1] Department of Agronomy, Iowa State University, Ames, IA 50011, USA
[2] Department of Crop and Soil Sciences, Washington State University, Pullman, WA 99164 USA
[3] USDA-ARS Wheat Health, Genetics, and Quality Research Unit, USDA-ARS, Pullman, WA 99164 USA
[4] USDA-ARS North Central Regional Plant Introduction Station, USDA-ARS, Ames, IA 50014 USA

An estimated 92% of maize harvested in the US is dependent on Corn Belt Dent germplasm (Smith et al., 2022), which harnesses about 2% of the known diversity of maize (Pollak, 2003). The Germplasm Enhancement of Maize (GEM) project is a public–private cooperative breeding program initiated to effectively increase the diversity of US maize germplasm by utilizing exotic, public, and proprietary maize germplasm. However, identifying and leveraging beneficial diversity is cumbersome without genomic data. To address this, two large doubled haploid (DH) populations were derived from double-double synthetic crosses to encapsulate the genetic diversity within the GEM material and the two major Corn-Belt heterotic groups. DH lines will be genotyped using a low-density genotype-by-sequencing strategy, while founder lines of the double-double crosses will be genotyped using high-density genome sequencing. This approach will allow for imputations with improved accuracies for genome-wide association studies and genomic selection. DH lines will be phenotyped for (1) agronomic traits per se and in testcross hybrid combination, (2) grain macromolecular composition using near-infrared spectroscopy (NIR), and (3) grain mineral concentrations using inductively coupled plasma-mass spectrometry (ICP-MS). This study will combine genotypic data with phenotypic data in an optimal training populations strategy to identify superior lines with rare tropical allelic diversity within GEM, and provide an invaluable community resource by making the GEM DH lines and their corresponding genomic and phenomic data publicly available.

Funding acknowledgement: United States Department of Agriculture (USDA)

## P214

## Construction of a high-density, sequence-indexed *Setaria viridis* NMU mutant population

(submitted by Greg Ziegler <gziegler@gmail.com>)
Full Author List: Ziegler, Greg[1]; Jiang, Hui[1]; Baxter, Ivan[1]
[1] Donald Danforth Plant Science Center; Saint Louis, MO 80120

*Setaria viridis* is a C4 grass species whose lifespan, stature, and genome size make it an ideal model for studying agronomically important traits that can then be exploited in maize breeding programs. We have developed a population of ~20,000 mutant families using N-nitroso-N-methylurea (NMU) to chemically induce mutations. The mutant families are being screened for visible phenotypes such as dwarfism, pale green tissue, and necrotic leaves which may indicate $CO_2$ sensitive and/or stomatal mutants. In addition, mutants are being screened using the Bellwether automated phenotyping facility to identify mutants with altered water-use-efficiency and growth phenotypes. To date, 900 M2 individuals have been sequenced at 30x coverage by the Joint Genome Institute. We have developed a filtering pipeline to remove false positive mutations resulting from the initial called SNPs which reduced the total mutations called for each variety from an average of ~23,000 to ~3,000 SNPs. Finally, we have developed an interactive web application for exploring the SNP dataset that can be used to find mutants with mutations in genes of interest or genes that have high impact mutations from a list of mutants exhibiting a common phenotype.

Funding acknowledgement: Department of Energy (DOE)

## P215

### Decoupling brace root development from phase change in sorghum: candidate genes revealed by GWAS

(submitted by Ashley Hostetler <ahende@udel.edu>)
Full Author List: Hostetler, Ashley N.[1 2]; Khangura, Rajdeep S.[3]; Dilkes, Brian P.[3]; Sparks, Erin E.[1 2]
[1] Division of Plant Sciences and Technology, University of Missouri, Columbia, MO, USA 65201
[2] Department of Plant and Soil Sciences, University of Delaware, Newark, DE, USA 19716
[3] Department of Biochemistry, Purdue University, West Lafayette, IN, USA 47907

Root lodging, a type of mechanical failure, negatively impacts the quality and quantity of crop yields. In maize, the presence of above ground nodal roots (called brace roots) is positively associated with lodging resistance. Sorghum, a close relative, also develops brace roots. We previously highlighted shared genetic controls between brace root development and phase change. Separately manipulating brace root development and phase change is necessary for breeding climate resilient crops. Using the Sorghum Association Panel (SAP) and Bioenergy Association Panel (BAP), we aimed to identify alleles that specifically impact brace root development but not phase change. Comparisons of plant height, flowering time, and brace root node number between populations demonstrated the existence of independent genetic regulation of brace root development. To identify genetic markers, genome wide association (GWA) analyses were performed within each population and regions of the genome were identified as significantly associated with each trait. Of the SNPs identified, candidate genes were prioritized if they were linked to alleles that only affected brace root node number and were identified in both populations. This identified alleles at genes that affected brace root development independent of phase change. These findings provide a basis for targeted studies aimed at uncovering the molecular basis of brace root development.

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## P216

### Designing AI models for genomic selection

(submitted by Karlene Negus <knegus@iastate.edu>)
Full Author List: Negus, Karlene L.[1]; Li, Xianran[2]; Welch, Stephen M.[3]; de los Campos, Gustavo[4]; Yu, Jianming[1]
[1] Department of Agronomy; Interdepartmental Genetics & Genomics, Iowa State University, Ames, IA 50011
[2] USDA-ARS, Wheat Health, Genetics & Quality Research, Pullman, WA 99164
[3] Department of Agronomy, Kansas State University, Manhattan, KS 66506
[4] Department of Plant, Soil and Microbial Sciences; Department of Epidemiology and Biostatistics; Institute for Quantitative Health Science and Engineering, Michigan State University, East Lansing, MI, 48824

Genomic selection is a key methodology for accelerating maize breeding. Genomic selection was developed to exploit the genotype-phenotype relationship by using genome-wide markers to predict estimates of breeding values and overcome the low genetic gains of marker-based selection for complex traits. However, the field has seen dramatic changes to the scale and diversity of available data since the development of early genomic selection models. While artificial intelligence (AI) genomic selection models have not yet consistently outperformed conventional regression-based strategies, AI models are rapidly evolving. We need to continue to assess the potential of new AI models if we intend to better capitalize on data-breakthroughs in the future. A key component of developing successful AI genomic selection models, which has thus far received limited focus, is fully contextualizing AI methodologies for breeding and genetics. Domain-aware modifications to AI models have driven the major successes of AI prediction in fields like natural language, chemistry, and medicine. Here we present an investigation into the role of key model components for designing better AI genomic selection models. Our approach looks at three major elements: overall model architecture, input data encoding strategy, and task specific layer structure. Transformers and transformer-derived architectures are the current state-of-the-art AI models. BERT (Bidirectional Encoder Representations from Transformers) and Jamba represent the early generation and recent generation transformer derivatives, respectively. We have implemented these architectures with out-of-the-box and domain-aware input encoding strategies, including allele dosage encoding, categorical SNP genotype encodings, and local-radial basis function encodings. Further, we compared standard and customized task-specific layers, including single-task regression and multi-task regression. With domain-aware modification we were able to achieve improved prediction accuracies over out-of-the-box model configurations. Adapting, rather than simple adopting, existing AI methods to leverage domain knowledge in genetics and breeding may bridge the gap between AI and genomic selection.

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

## P217

## Dissecting the genetic architecture of cold acclimation and freezing tolerance in Tripsacum using eQTL mapping

(submitted by Lara Brindisi <lara.brindisi@usda.gov>)
Full Author List: Brindisi, Lara J.[1 2]; Gault, Christy[2]; Costich, Denise[2]; Buckler, Edward S.[1 2]
[1] USDA-ARS, Ithaca, NY, USA 14853
[2] Institute for Genomic Diversity, Cornell University, Ithaca, NY USA 14853

Maize (Zea mays subsp. mays) is very sensitive to low temperatures, which restricts cultivation to late spring once the threat of frost subsides in North America. Introducing cold and freezing tolerance into maize would allow for earlier planting dates, enabling capture of more solar radiation and soil nitrogen available in early spring. These benefits have the potential to improve yield through increased radiation use efficiency (RUE) and reduce nitrous oxide emissions with increased nitrogen use efficiency (NUE). Eastern gammagrass (Tripsacum dactyloides) is a close relative of maize that exhibits robust growth in temperate North America and thus serves as a rich source for potential cold tolerant alleles that are transferable to maize. This study investigates the genetic basis of cold acclimation and freezing responses in an F2 population (n=288), derived from T. dactyloides parents originating from different latitudes with varying degrees of cold tolerance. By employing expression quantitative trait locus (eQTL) mapping, we aim to identify key genes and expression patterns associated with tolerance to low temperatures. Such advancements would enhance maize productivity and reduce greenhouse gas emissions in agriculture.

Funding acknowledgement: United States Department of Agriculture (USDA)

## P218

## Dissection of genetic basis of maize resistance to Gibberella Ear Rot and its associated seed microbiome

(submitted by Xiquan Gao <xgao@njau.edu.cn>)
Full Author List: Zhai, Kaihui[1 2 3]; Zhang, Yingying[1 2 3]; Zhao, Zian[1 2 3]; Li, Xin[1 2 3]; Wang, Qing[1 2 3]; Gao, Xiquan[1 2 3]
[1] State Key Laboratory for Crop Genetics & Germplasm Enhancement and Utilization
[2] Collaborative Innovation Center for Modern Crop Production co-sponsored by Province and Ministry
[3] College of Agriculture, Nanjing Agricultural University

With long-term coevolution, crops have established either mutualistic or antagonistic relationship with beneficial microbes or phytopathogens, respectively. Maize (*Zea mays* L.) diseases caused by *Fusarium graminearum,* including Gibberella Ear Rot (GER) and Gibberella Stalk Rot (GSR), are among the major concerns of maize production, which greatly impact maize yield and quality. However, the genetic basis underlying the mechanisms regulating both the resistance to *F. graminearum* and the associated microbiome remain largely unexplored. A joint analysis of genome wide association study (GWAS) and microbiome analysis using a population consisting of 250 maize inbred lines was deployed to identify the genetic loci for GER resistance and the community of endophytic microbiome associated with the resistance to GER. In total, 332 significant SNPs and 115 candidate genes associated with GER resistance were identified. Haplotype analysis of several candidate genes showed that there was significant difference between resistant and susceptible groups. The community complexity of seed endophytes in susceptible group was more intricate than resistant group. Furthermore, 11 OTUs belonging to 8 genera of seed endophytes showed significant difference between two groups. GWAS analysis using significant OTUs identified 211 significant SNPs associated with 65 genes. The candidate endophyte strain was identified and functionally validated. In summary, this work identified potential genetic loci and candidate genes regulating resistance to *F. graminearum* and that reshaping the microbiome community potentially impacting resistance to GER, which provide promising alternative strategies to understand the host-microbiome-pathogen interaction, thereby, to benefit maize disease resistance breeding.

Funding acknowledgement: NSFC

## P219

### Engineering synthetic apomixis in maize

(submitted by Nina Chumak <nina.chumak@botinst.uzh.ch>)
Full Author List: Chumak, Nina[1]; Conner, Joann A.[2]; Ozias-Akins, Peggy[2]; Grossniklaus, Ueli[1]
[1] University of Zurich, Department of Plant and Microbial Biology, Zollikerstrasse 107, Zurich, CH-8008, Switzerland
[2] University of Georgia, Department of Horticulture, 2360 Rainwater Road, Tifton, GA 31793-5766, USA

Apomixis, asexual reproduction through seeds, is a reproductive strategy occurring in more than 400 species of flowering plants, but does not occur in the major crop species. Since apomictic plants reproduce clonally, their offspring is genetically identical to the mother plant. If engineered in crop species, apomixis would allow the maintenance of highly genetically heterozygous genotypes. For instance, it could be used to preserve the genotype of $F_1$ hybrids over many generations without losing hybrid vigor. Current strategies to engineer apomixis require mutations in three to five meiotic genes causing a 'mitosis instead of meiosis' or MiMe phenotype, combined with the expression of a *BABY BOOM-LIKE* (*BBML*) transgene in the egg cell to initiated parthenogenesis. Although this strategy provided good results in rice, its implementation in current breeding schemes might be challenging due to requirement to manipulate multiple genes. Thus, simpler approaches to engineer apomixis may be advantageous.We performed genetic screens to identify mutations mimicking components of apomixis in maize and will present our progress in combining on such mutation with a *BBML* transgene to generate clonal seeds in maize.

Funding acknowledgement: SNF

## P220

### Environmental and genetic factors underlying maize cuticular wax accumulation under drought stress

(submitted by Matthew Wendt <wendtmat@iastate.edu>)
Full Author List: Wendt, Matthew M.[1]; Hattery, Travis J.[1]; Chen, Keting[1]; Granados-Nava, Karen[1 2]; Schroeder, Elly[1 3]; Myers, Bryn[1 2]; Moore, Riley[1 4]; Loneman, Derek[1]; Claussen, Reid[1]; Gilbert, Amanda[1]; Garfin, Jacob[5]; Bjerklie, Dane[5]; Lauter, Nick[6 7]; Hirsch, Candice N.[5]; Yandeau-Nelson, Marna D.[1]
[1] Department of Genetics, Development, and Cell Biology, Iowa State University; Ames, Iowa 50011, USA
[2] Undergraduate Genetics Major, College of Liberal Arts and Science, Iowa State University; Ames, Iowa 50011, USA
[3] Undergraduate Kinesiology Major, College of Human Sciences, Iowa State University; Ames, Iowa 50011, USA
[4] Undergraduate Biology Major, College of Liberal Arts and Sciences, Iowa State University; Ames, Iowa 50011, USA
[5] Department of Agronomy and Plant Genetics, University of Minnesota; Minneapolis, Minnesota 55455, USA
[6] Department of Plant Pathology and Microbiology, Iowa State University; Ames, Iowa 50011, USA
[7] USDA-ARS Corn Insects and Crop Genetics Research Unit, Iowa State University; Ames, Iowa 50011, USA

Climate change will drive changes in global temperature for the foreseeable future, which is likely to prolong drought events. One mechanism by which plants can partially ameliorate the effects of drought and other stresses is via the hydrophobic cuticle, composed of cutin and very long-chain waxes, which coats the epidermis of aerial plant organs. Both the quantity and types of cuticular waxes contribute to the degree of cuticle hydrophobicity, and thereby efficiency as a barrier to non-stomatal water-loss. We've previously shown how weather events such as drought or solar radiation are associated with cuticular wax profile. In this study, we are analyzing both the genetic architecture and environmental response genes underlying cuticular wax composition. We first analyzed cuticular wax composition data (45 metabolites) on silks across 448 diverse maize inbred lines of the Wisconsin Diversity Pane using high-dimensional seemingly-unrelated multivariate modeling to determine the relative importance of multiple weather parameters during the growing season on cuticular wax accumulation on maize silks. We then used these same weather parameters to estimate the contribution of specific alleles to both genotype and genotype-by-weather effect estimates by multivariate GWAS, identifying candidate genes that explain variation in waxes across abiotic environmental gradients. Characterization of genes that contribute to the genetic architecture of cuticular waxes between environments will enable the design of weather resilient or "climate-smart" plants that remain healthy and productive under the environmental stresses associated with climate change.

Funding acknowledgement: National Science Foundation (NSF)

## P221

## Evaluating early vigor in maize genotypes under pythium root rot stress: A pilot experiment

(submitted by Joseph Romano <jromano@udel.edu>)
Full Author List: Romano, Joseph[1]; Wright, Erin[1]; Subedi, Manoj[1]; Kim, Jeonghwa[1]; Betts, Alyssa[1]; Mu, Qi[1]
[1] Department of Plant and Soil Sciences, University of Delaware, Newark, DE, 19716

*Pythium* Root Rot (PRR) is a disease caused by several species of *Pythium* and *Globisporangium* oomycetes. These pathogens are soil borne, and cause millions of dollars in annual economic losses to corn (*Zea mays* L.) production across the United States. Symptoms of the disease include stunted growth, necrotic mesocotyl tissue, and damping off. Screening maize genotypes under diverse genetic backgrounds is critical for improving their resistance to PRR disease. Here, we conducted a pilot greenhouse-based experiment to screen three maize genotypes under inbred, landrace, and hybrid genetic backgrounds for resistance to PRR. The experimental trials were conducted with three pathogen treatments (*P. inflatum*, *G. sylvaticum*, and *P. graminicola*) and a control. Disease severity was evaluated by measuring seedling survival and growth rates, shoot and root weight, and total shoot length. Our findings show that the tested maize genotypes differed in their performance against PRR species. Additionally, the severity of different PRR species varied, denoting differences in aggressiveness among the PRR pathogens. These observations suggest that genetic variability for PRR resistance exists among maize genotypes. Collectively, our pilot experiment indicates that identifying PRR-resistant maize lines is feasible, offering promising opportunities for future maize breeding efforts.

Funding acknowledgement: United States Department of Agriculture (USDA)

## P222

## Exploring trade-offs between drought and cold tolerance in *Tripsacum dactyloides*.

(submitted by Beatrice Konadu <bak234@cornell.edu>)
Full Author List: Konadu, Beatrice[1]; Lai, Wei-Yun[2]; Stitzer, Michelle[2]; El-Walid, Mohamed[1]; Hsu, Sheng-Kai[2]; Romay, Cinta[2]; Buckler, Edward[1 2 3]
[1] Section of Plant Breeding and Genetics, Cornell University, Ithaca, NY 14853
[2] Institute for Genomic Diversity, Cornell University, Ithaca, NY 14853
[3] USDA-ARS; Ithaca, NY 14853

As climate change intensifies, crops are increasingly exposed to unpredictable environmental stresses that challenge their survival and productivity. Drought and extreme temperatures are two of the most disruptive climatic stresses for plants, severely affecting growth, reproduction, and survival. *Tripsacum dactyloides*, a close relative of maize, demonstrates unique adaptive traits that may hold keys to improving stress tolerance in related species. Understanding the physiological and genetic tradeoffs between drought and cold tolerance in *Tripsacum dactyloides* is critical for developing resilient crops. This study aims to explore the genetic mechanisms underlying drought and cold tolerance in *T. dactyloides*, examining how traits that enhance survival under one stress may compromise resilience to the other. This will be done by employing an expression Quantitative Trait Locus (eQTL) mapping approach to identify genomic regions associated with tolerance to both drought and cold stress. This research aims to pinpoint loci where genetic variation and specific pathways contribute to stress response, highlighting potential tradeoffs where increased tolerance to one type of stress may reduce/increase resilience to another.

Funding acknowledgement: United States Department of Agriculture (USDA)

## P223

### *Expression variation in Glossy15 is associated with maize harvest index.*

(submitted by Steve Moose <smoose@illinois.edu>)
Full Author List: Rai, Nandan Maruti[1]; Zhang, Wei[1 2]; Moose, Steve[1]
[1] University of Illinois at Urbana-Champaign, 61801
[2] Ultragenyx, Somerville, Massachusetts, 02143

Maize grain yields in the US Corn Belt have risen 6-fold during the past century, due in part to increasing harvest index, the ratio of harvested grain to total biomass. Although larger ears with more and heavier kernels certainly contribute to higher harvest index, the potential influence of changes in vegetative biomass has not been directly explored. We demonstrate here that variation in the expression of the APETALA2-class transcription factor *Glossy15* (*Gl15*), a key regulator of vegetative phase change, also modulates harvest index. Historical maize breeding has shortened the duration of the juvenile vegetative phase, which is also associated with fewer vegetative nodes. Two classes of functional *Gl15* haplotypes exist in maize germplasm, those with a second *microRNA172* (*miR172*) target site that are more weakly expressed compared to haplotypes that lack the secondary *miR172* site. The weaker *Gl15* haplotypes are associated with a shorter juvenile phase and higher harvest index in populations of maize hybrids, although the two classes of haplotypes appear to be under balancing selection. Cis-genic introduction of a native *Gl15* haplotype lacking the secondary *miR172* target site increases *Gl15* expression, delays vegetative phase change, and reduces harvest index in multiple hybrid backgrounds. Conversely, near-isogenic hybrids homozygous for *gl15* loss-of-function mutations exhibit higher harvest index via reductions in stalk biomass while maintaining grain yield. These findings indicate that selection for a shorter juvenile phase, mediated by native *Gl15* alleles with greater sensitivity to *miR172*-mediated repression, offers a novel opportunity to further improve maize harvest index.

Gene / Gene Models described: *glossy15*; Zm00001eb387280
Funding acknowledgement: United States Department of Agriculture (USDA)

## P224

### Field-scale and high-throughput maize leaf angle characterization using stereo vision and deep learning

(submitted by Xuan Liu <xuanliu@iastate.edu>)
Full Author List: Liu, Xuan[1]; Raj, Aditya[1]; Xiang, Lirong[1 3]; Yu, Jianming[2]; Schnable, Patrick[2]; Tang, Lie[1]
[1] Department of Agricultural and Biosystems Engineering, Iowa State University, Ames, Iowa 50011
[2] Department of Agronomy, Iowa State University, Ames, Iowa 50011
[3] Department of Biological and Agricultural Engineering, North Carolina State University, Raleigh, North Carolina 27695

Maize leaf angle is a critical plant architectural trait as it plays an important role in light interception that defines plant canopy's photosynthetic capacity. To automate field-scale maize leaf angle characterization, a mobile platform, Phenobot, equipped with a highly competent sensing system was developed to generate large data sets of plant architecture traits for tall stature crops like corn during their entire growing season. The platform features five tiers of customized PhenoStereo 3D cameras positioned at varying heights to expand the vertical field of view. The customized PhenoStereo cameras are equipped with strobe lights to ensure the quality of the images despite the vibrations caused by the uneven field and the variation of outdoor lighting conditions. An automated image processing pipeline based on deep learning was developed to detect leaf collars in color images and measure leaf angles in reconstructed 3D models to conduct a high-throughput leaf angle characterization. 3D reconstruction is essential for the proposed pipeline to produce satisfactory measurement accuracy and efficiency. After comparison with multiple state-of-the-art algorithms, CREStereo algorithm was used for stereo matching due to its excellent performance in both accuracy and speed. The relative positions between different tiers of stereo cameras were used to eliminate duplicated leaf angle measurements from the images captured by the adjacent stereo cameras. In addition, the overlaps between sequential images were removed using wheel encoder signals and tracking plant stalks. The results demonstrated that the proposed system can effectively perform large-scale, field-based, high-throughput phenotyping of maize leaf angles. This advancement allows for the acquisition and analysis of field scale datasets, helping plant scientists to analyze genetic associations to different leaf angle formations.

Funding acknowledgement: National Science Foundation (NSF)

## P225 @caneryz

### Fine mapping of QTLs for candidate genes responsible for embryogenic callus induction in maize

(submitted by Caner Yavuz <cyavuz2@unl.edu>)
Full Author List: Yavuz, Caner[1 2]; Sun, Guangchao[1 3]; Sato, Shirley J.[1]; Turkus, Jonathan D.[1 2]; Schnable, James C.[1 2]; Clemente, Tom Elmo[1]
[1] Department of Agronomy and Horticulture, University of Nebraska-Lincoln, Lincoln, NE, USA 68588
[2] Center for Plant Science Innovation, University of Nebraska-Lincoln, Lincoln, NE, USA 68588
[3] Maize Research Institute, Sichuan Agricultural University, Chengdu, Sichuan, China

Achieving high rates of callus induction is crucial for various tissue culture applications in plants, including plant transformation and genome editing. In maize, the ability of immature embryos to generate embryogenic calli is heavily impacted by genotype, media composition, and explant type. Potential candidate genes responsible for natural variation in callus induction and regeneration remains underexplored and has yet to be thoroughly investigated. Two critical morphogenic regulators BABY BOOM (BBM) and WUSCHEL (WUS), which have been linked to increased regeneration via transgenic investigation are located on on chromosomes 5 and 6, respectively, but it remains unclear whether natural variation at these loci contributes to the observed variability in callus induction among maize genotypes. In this study, we developed an $F_2$ population from a cross between B73 (showing lower rate of embryogenic callus response) and CML216 (showing higher rate of embryogenic callus induction) and found that induction of embryogenic callus segregated at a 1:3 ratio. QTL mapping using using bulked segregant analysis (BSA) and tunable genotyping-by-sequencing (tGBS) data revealed two QTLs on two chromosomes, 1 and 2, regions not previously associated with major embryogenic callus formation related regulatory genes, such as BBM, WUS. We are now using a fine mapping approach to investigate natural variation for embryogenic callus induction in a backcross population ($BC_1F_1$) using KASP markers spanning the previously identified QTL on chromosomes 1 and 2. The finding of this research will significantly enhance our understanding of the role of various potential morphogenic regulators in embryogenic callus induction in maize.

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## P226

### From field to future: Environmental impacts on parental and progeny corn traits

(submitted by Donielle Brottlund <brottlu1@msu.edu>)
Full Author List: Brottlund, Donielle C[1]
[1] Michigan State University; East Lansing, MI, 48824

Plants' ability to adapt to environmental conditions and pass traits to subsequent generations is a cornerstone of natural selection. While it is well-documented that environmental factors influence plant growth, the extent to which these effects on parental plants impact their offspring remains less understood. Genotype by environmental interaction is critical for us to understand because it significantly impacts the growth, yield, and overall performance of a crop. This study investigates how the growth environment of parental plants affects the performance of their progeny using field corn hybrids as a model system.We considered nine field corn hybrids, with parental inbred seeds originating from Michigan. These seeds were distributed to diverse environments across the United States, including Iowa, Missouri (two locations), Wisconsin, Illinois, Georgia, Florida, and Michigan. These inbreds were grown and cross pollinated before sending the seeds back to Michigan. To minimize confounding effects, hybrid seeds were weighed and sorted by size, and only medium-sized kernels were used for a replicated common garden experiment in East Lansing, MI.Phenotypic traits including growth characteristics, kernel weights, flowering, and plant heights of the progeny plants grown in East Lansing were measured to assess the impact of environmental variability in seed production sites on the resulting hybrid seed. By analyzing offspring grown in uniform conditions, we aim to isolate and understand the environmental effects experienced by the parents that influence growth in the next generation. This research advances our understanding of transgenerational environmental influences in crop systems and provides insights into optimizing seed production and hybrid performance across variable agricultural landscapes.

# P227

## From point clouds to canopy architecture insights: High-throughput trait extraction from UGV-based LIDAR in maize

(submitted by Luke Gregory <lmg342@cornell.edu>)
Full Author List: Gregory, Luke M.[1]; Lee, Seungjoon[2]; Du, Ruiming[3]; Han, Tao[4]; Farmer, Erin[1]; Wickes-Do, Liam[1]; Michael, Peter[5]; Gu, Zeqi[5]; Yan, Ruyu[5]; Lepak, Nick[6]; Romay, Cinta[7]; Buckler, Edward S.[1 6 7]; Davis, Abe[5]; Xu, Xiangtao[4]; Jiang, Yu[3]; Sun, Ying[2]; Gore, Michael A.[1]
[1] Plant Breeding and Genetics Section, School of Integrative Plant Science, Cornell University, Ithaca, New York 14853, USA
[2] Soil and Crop Sciences Section, School of Integrative Plant Science, Cornell University, Ithaca, NY 14853, USA
[3] Horticulture Section, School of Integrative Plant Science, Cornell University, Geneva, NY 14456, USA
[4] Department of Ecology and Evolutionary Biology, Cornell University, 215 Tower Rd., Ithaca, NY 14853, USA
[5] Department of Computer Science, Cornell University, Ithaca, New York 14853, USA
[6] United States Department of Agriculture-Agricultural Research Service, Robert W. Holley Center for Agriculture and Health, Ithaca, New York 14853, USA
[7] Institute for Genomic Diversity, Cornell University, Ithaca, New York 14853, USA

A maize canopy fulfills multiple structural functions to support physiological and agronomic outcomes. These include the interception and distribution of light to enhance photosynthetic efficiency, creating mechanical support through robust stalks to withstand lodging, and the arrangement and density of leaves, which shape the canopy microclimate. Each of these processes plays an important role in determining maize yield, whether directly contributing to grain filling or altering pest/disease dynamics. Understanding canopy structure is essential for optimizing these physiological processes within a specific growth environment, a goal that can be achieved through breeding and management practices. In this work, we leveraged unmanned ground vehicle (UGV)-based LiDAR to generate low-density point clouds, scanning hundreds of two-row maize hybrid plots in the Genomes To Fields (G2F) experimental site in Aurora, NY. This data enables the extraction of key canopy architecture traits essential for characterizing structural features of the canopy. Our approach integrates computational geometry and machine learning techniques to denoise, segment, and analyze the low-density point clouds, ensuring consistent and programmatically scalable trait extraction across genotypes. High-throughput analyses were conducted to quantify traits such as leaf angle distribution (and its vertical profile), leaf count, and leaf area index. Additionally, we derived density-based metrics partitioned by canopy height and plot width, revealing patterns of biomass accumulation and spatial heterogeneity among genotypes. Preliminary results across a multiyear collection period reveal variation in canopy traits across genotypes, highlighting the potential for this approach to enhance our understanding of genotype-by-environment interactions. By integrating UGV-based phenotyping with existing G2F datasets, this study offers new opportunities to identify architectural traits associated with yield and stress resilience. Our findings highlight the utility of UGV-based low-density LiDAR systems for high-throughput phenotyping in crop breeding programs and emphasize the value of integrating multiscale data to advance predictive analytics in maize improvement.

Funding acknowledgement: United States Department of Agriculture (USDA), USDA - AFRI competitive grant

## P228

### *Genetic analyses of novel inflorescence architecture traits in broomcorn*

(submitted by Dikshya Sapkota <dikshya2@illinois.edu>)
Full Author List: Sapkota, Dikshya[1]; Dhakal, Smit[1 2]; Bubert, Jessica[1 3]; Studer, Anthony J.[1]; Moose, Stephen P.[1]
[1] Department of Crop Sciences, University of Illinois at Urbana-Champaign, 61801
[2] PowerPollen, Ames, IA, 50010
[3] AgriReliant Genetics, Esmond, Illinois, 60129

Broomcorn (*Sorghum vulgare* var. *technicum)* describes historically important varieties of sorghum specifically selected for long panicle branches used in making natural fiber brooms. Ideally, broomcorn panicles have branches that are at least 50 cm (20 inches) in length, minimal internode elongation between branches, and formation of spikelet pair meristems only at the tips of the branches. This panicle morphology is well-suited for either hand or mechanical harvesting by cutting just below the peduncle, after which seeds are removed to produce fibers ready for broom production. The pedicels and trichomes at the ends of the fibers also capture dust particles more effectively than smooth plastic fibers. Thus, broomcorn is an interesting example of artificial selection for novel features of grass inflorescence architecture. Central Illinois was the historic center for broomcorn and broom production in the United States, where the University of Illinois maintains broomcorn germplasm and continues breeding efforts. SNP genotyping of broomcorn germplasm reveals two major subpopulations that cluster within the ancestral "bicolor" racial group of broader sorghum diversity, indicative of ancient origin. Analysis of a mapping population derived from crossing broomcorn with the A1-Tx623 grain sorghum parent demonstrated independent inheritance of broomcorn traits (panicle length, degree of branching, proportion of branches bearing seeds) and plant height or flowering time. Panicle length displayed a complex genetic architecture, whereas degree of branching was controlled by two major effect QTL. One of these QTL was linked to a fertility restorer gene for A1 sterile cytoplasm, knowledge which has been applied to overcome the historical difficulty in developing male-sterile broomcorn varieties. Fine-mapping of major effect QTL is in progress to identify the genes that program the unique inflorescence architecture of broomcorn and inform future efforts to improve the crop as a source of strong, renewable, plant-based fiber.

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

## P229

### Genetic analysis of fertility restoration in cytoplasmic male sterility-C type using WI-SS-MAGIC population and ex-PVP maize lines

(submitted by John Searl <jsearl@wisc.edu>)
Full Author List: Searl, John C[1]; de Leon, Natalia[1]; Kaeppler, Shawn M[1]
[1] Department of Plant and Agroecosystem Sciences, University of Wisconsin, 1575 Linden Dr., Madison, WI 53706

Cytoplasmic Male Sterility (CMS) is a widely used system for the cost-effective production of maize (*Zea mays* L.) hybrid seed in the commercial sector. Natural restorers of fertility for the C-Type cytoplasm (CMS-C) exist in Midwest-dent germplasm. Understanding the sources of restorers and their inheritance will facilitate the breeding of stable male-sterile seed parents and the development of fertile hybrids for commercial production. In this study, we evaluated 475 inbreds from the Wisconsin Stiff Stalk Multiparent Advanced Generation Inter-Cross (MAGIC) mapping population in addition to 100 priority ex-PVP inbreds. The goal of the study was to genetically map restorer genes in the Stiff Stalk background and screen a more diverse array of inbreds for fertility in the CMS-C cytoplasm. We crossed all inbreds to a common CMS-C tester, resulting in 575 $F_1$ hybrids. These hybrids were grown in a randomized block design with two replicates during the summer of 2024 and assessed for fertility. Linkage mapping within the MAGIC population revealed four quantitative trait loci (QTL) associated with fertility restoration, including the well-known candidate gene *restorer of fertility 4* (*rf4*). Our findings enhance the understanding of the genetic control of fertility restoration in CMS-C, offering hybrid seed producers strategies to reduce costs and improve production efficiency.

Funding acknowledgement: Gabelman-Shippo Wisconsin Distinguished Graduate Fellowship, USDA-Hatch

## P230

## Genetic architecture underlying mean and plasticity of kernel traits in maize highlights independent control and key loci

(submitted by Shalma Maman <Shalma.Maman@jacks.sdstate.edu>)
Full Author List: Maman, Shalma[1]; Assefa, Ermias[1]; Mural, Ravi V[1]; Schnable, James C.[2]
[1] Department of Agronomy, Horticulture and Plant Science, South Dakota State University, Brookings, SD, United States 57006
[2] 3Department of Agronomy and Horticulture, University of Nebraska–Lincoln, Lincoln, Nebraska, USA

Compositional traits are critical determinants of grain quality and play a significant role in food production and biofuel potential. In the United States, maize breeding programs have historically prioritized yield over quality traits, including oil and starch content, which are essential for improving biofuel production. Understanding the genetic and environmental factors driving variation in compositional traits is vital for realigning breeding goals to enhance grain quality and biofuel feedstock potential. This study explores the genetic control underlying trait mean, linear plasticity, and nonlinear plasticity across six kernel traits in maize. Using two partially overlapping maize association panels and the US-NAM population evaluated in diverse environments, we quantified trait means and plasticity through Bayesian Finlay-Wilkinson regression. Genome-wide association studies (GWAS) with high-density markers and linkage disequilibrium-based peak merging identified high-confidence marker-trait associations, revealing independent genetic control for linear plasticity. We identified 19 genetic peaks associated with trait means, 8 with plasticity, and 7 influencing both parameters, with a robust confidence threshold (≥20 RMIP). Notably, DGAT1-2 (ln1), a key gene in triacylglycerol (TAG) biosynthesis, was linked to phenotypic plasticity, with the rarer allele (T) exhibiting contrasting effects on mean expression and plasticity. These findings uncover a complex genetic network involving pleiotropy, multiple alleles, and GxE interactions, advancing our understanding of kernel trait variability. By pinpointing loci linked to oil and starch traits, this study offers valuable targets for breeding programs aimed at enhancing maize quality for biofuel production, alongside traditional agricultural uses.

## P231

## Genetic dissection and characterization of yield component traits in maize

(submitted by Waqar Ali <wali3@huskers.unl.edu>)
Full Author List: Ali, Waqar[1 2]; Davis, Jensina[1 2]; Shrestha, Nikee[1 2]; Turkus, Jon[1 2]; Schnable, James C.[1 2]
[1] Department of Agronomy and Horticulture, University of Nebraska-Lincoln, Lincoln, NE, USA
[2] Center for Plant Science Innovation, University of Nebraska-Lincoln, Lincoln, NE, USA

Being a composite trait, yield is determined by various factors including the kernels row number, kernels per row, 100 kernel weight, and yield related traits like ear length, ear width, ear weight and kernel weight. Here we sought to identify genes with roles in specifying variation in specific yield component traits. We generated and utilized a dataset comprising data from approximately 16 thousand hand phenotyped ears from 714 corn genotypes drawn the Wisconsin Diversity Panel, scored in two replicated field trials conducted in different years. After data curation, we performed the spatial correction to correct plot level values and then calculated the Best Linear Unbiased Estimates (BLUEs) for these yield related traits. A set of 13.6 million high density SNPs markers accounting for 5 % MAF and 2% heterozygosity, was generated to run association study to capture the significant associations with above mentioned yield and related traits. A resampling-FarmCPU GWAS was run to identify genetic variants linked to the above-mentioned yield components and yield related components. We got 15 GWAS hits associated with 100 kernel weight, kernel row number, kernels per row, ear length and ear width at RMIP > 0.2. These 15 GWAS hits were individually associated with between X and Y potential candidate genes, considering all gene models location within 50 kilobases of the trait associated SNP. These are the preliminary results of ongoing analysis. The linkage disequilibrium analysis will help to determine potential candidates based on the LD association of GWAS associated markers with nearby markers. The stability of GWAS identified associations will be strengthened by cross-validating the results of existing GWAS associations by using a subset of inbreds grown across years of 2022 and 2023 at diverse locations including Lincoln, Ames, Crawfordville, Missouri valley, and Scottsbluff. Based on the findings, we will prioritize the candidate genes and validate them by growing their CRISPR-edits mutants in greenhouse.

Funding acknowledgement: Higher Education Commission of Pakistan

## P232

# Genetic diversity and inter-trait relationship of tropical extra-early maturing quality proteinmaize inbred lines under low soil nitrogen stress

(submitted by Pearl Abu <plabu@wacci.ug.edu.gh>)
Full Author List: Abu, Pearl[1]; Badu-Apraku, Baffour[2]; Ifie, Beatrice E.[1]; Tongoona, Pangirayi[1]; Melomey, Leander D.[1]; Offei, Samuel K.[1]
[1] West Africa Centre for Crop Improvement, University of Ghana, Legon, Ghana,
[2] International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria

Information on the genetic diversity, population structure, and trait associations of germplasm resources is crucial for predicting hybrid performance. The objective of this study was to dissect the genetic diversity and population structure of extra-early yellow and orange quality protein maize (QPM) inbred lines and identify secondary traits for indirect selection for enhanced grain yield under low-soil nitrogen (LN). One hundred and ten inbred lines were assessed under LN (30 kg ha -1) and assayed for tryptophan content. The lines were genotyped using 2500 single nucleotide polymorphism (SNP) markers. Majority (85.4%) of the inbred lines exhibited wide pairwise genetic distances between 0.4801 and 0.600. Genetic distances were wider between yellow and orange endosperm lines and predicted high heterosis in crosses between parents of different endosperm colours. The unweighted pair group method with arithmetic mean (UPGMA) and the admixture model-based population structure method both grouped the lines into five clusters. The clustering was based on endosperm colour, pedigree, and selection history but not on LN tolerance or tryptophan content. Genotype by trait biplot analysis revealed association of grain yield with plant height and ear height. TZEEQI 394 and TZEEIORQ 73A had high expressivity for these traits. Indirect selection for high grain yield among the inbred lines could be achieved using plant and ear heights as selection criteria. The wide genetic variability observed in this study suggested that the inbred lines could be important sources of beneficial alleles for LN breeding programs in sub-Saharan Africa.

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## P233

## Genome-wide dissection of leaf angle variation across the canopy in maize

(submitted by Jake Hinrichsen <jhinrich@iastate.edu>)
Full Author List: Yu, Jiaming[1]; Tang, Lie[2]; Negus, Karlene[1]; Liu, Xuan[2]; Raj, Aditya[2]; Zhang, Ruqiang[3]; Char, Si Nian[4]; Alladassi, Boris M.E.[1]; Li, Dongdong[1]; Schoenbaum, Gregory R.[1]; Hinrichsen, Jacob T.[1]; Evans, Lukas J.[3]; Li, Xianran[5]; Bang-Jensen, Valerie[6]; Lubkowitz, Mark[6]; Yang, Bing[4]; Scanlon, Michael J.[3]
[1] Department of Agronomy, Iowa State University, Ames, IA, USA 50011
[2] Department of Agricultural and Biosystems Engineering, Ames, IA, USA 50011
[3] Plant Biology Section, School of Integrative Plant Science, Cornell University, Ithaca, NY, USA 14853
[4] Division of Plant Sciences, University of Missouri, Columbia, MO, USA 65201
[5] USDA-ARS, Wheat Health, Genetics, and Quality Research Unit, Pullman, WA, USA 99164
[6] Saint Michael's College, Colchester, VT, USA 05439

Leaf angle is a strategic component of plant architecture, and an important area of plant research that interconnects fundamental research on the mechanisms of boundary formation during plant development and agriculture production through improved crop canopy for increased productivity. Due to lack of reliable, automated strategies for measurement in high-throughput, leaf angle has been typically measured on a single leaf per plant in large-scale genetic studies. Several mutations affecting maize leaf angle were cloned and characterized, and genetic and transcriptomic analyses identified additional candidate genes implicated in ligule-auricle development. There remains a huge gap between known developmental genes and their contributions to the natural, phenotypic variation observed in diverse maize accessions. Our analysis has demonstrated that diverse maize inbreds are extremely polymorphic for this “within-plant, leaf angle variation” phenotype. Our overall project goal is to enrich the fundamental understanding of the genetic control of leaf angle variation across the canopy in maize and to provide mechanistic insights into genetic manipulation of plant architecture for continued crop improvement. We are working on four connected aims in this project. Aim 1: Genome-wide identification of genes underlying leaf angle variation across the canopy. This is facilitated by high-throughput phenotyping with a PhenoBot system to quantify multiple leaf angles at different nodes. Aim 2: Single-cell RNA sequencing analyses of genes underlying leaf angle variation at two contrasting canopy levels. Aim 3: Functional analyses of genes underlying leaf angle variation through CRISPR/Cas9-based gene editing in maize inbreds with different leaf angle types. Aim 4: Develop educational materials for K-12 teachers to create teaching gardens that help incorporate plant biology content into their curriculum. In addition to cross-training of postdocs and students, we will specifically develop and disseminate grade-level text sets and accompanying seeds for the creation of teaching gardens on school grounds.

Funding acknowledgement: National Science Foundation (NSF)

## P234

## Genomic prediction insights across haploid and diploid levels in maize

(submitted by Yu-Ru Chen <yuruchen@iastate.edu>)
Full Author List: Chen, Yu-Ru[1]; Fakude, Mercy[1]; Frei, Ursula K[1]; Lübberstedt, Thomas[1]
[1] Department of Agronomy, Iowa State University, Ames, IA 50011-1051, USA

The production of haploid plants at an extremely large scale in maize, followed by the generation of doubled haploids (DHs) in field nurseries, offers a transformative approach to maize breeding. By enabling selection and mating at the haploid stage, breeding cycle times can be significantly shortened, leading to increased genetic gains. With the successful integration of spontaneous haploid genome doubling in breeding populations and the reduced cost of genome-wide SNP genotyping, genomic prediction in haploid plants has become a viable strategy for accelerating breeding programs. In this study, the population of maize BS39 DHs was utilized to evaluate the predictive ability (PA) of seven agronomic traits across haploid and diploid ploidy levels by the rrBLUP model. Results revealed that the PAs for plant height, ear height, flag leaf width, and tassel branch exceeded 0.3 and improved from the haploid to the diploid level. Conversely, traits such as flag leaf length, spike length, and tassel length exhibited a declining PA trend from haploid to diploid stages. These findings highlight the feasibility of implementing genomic prediction at the haploid stage for maize breeding, while its effectiveness varies depending on the traits.

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## P235

### Genomic selection: Essence, applications, and prospects

(submitted by Jianming Yu <jmyu@iastate.edu>)
Full Author List: Escamilla, Diana M.[1]; Li, Dongdong[1]; Negus, Karlene L.[1]; Kappelmann, Kiara L.[1]; Kusmec, Aaron[1 2]; Vanous, Adam E.[3]; Schnable, Patrick S.[1]; Li, Xianran[4]; Yu, Jianming[1]
[1] Department of Agronomy, Iowa State University, Ames, IA, USA 50011
[2] Department of Agronomy, Kansas State University, Manhattan, KS, USA 66506
[3] USDA-ARS, North Central Regional Plant Introduction Station, Ames, IA, USA 50014
[4] USDA-ARS, Wheat Health, Genetics, and Quality Research Unit, Pullman, WA, USA 99164

Genomic selection (GS) emerged as a key part of the solution to ensure food supply for the growing human population, thanks to advances in genotyping and other enabling technologies and improved understanding of the genotype-phenotype relationship in quantitative genetics. GS is a breeding strategy to predict the genotypic values of individuals for selection using their genotypic data and a trained model. It includes four major steps: training population design, model building, prediction, and selection. GS revises the traditional breeding process by assigning phenotyping a new role of generating data for the building of prediction models. The increased capacity of GS to evaluate more individuals, in combination with shorter breeding cycle times, has led to a wider adoption in plant breeding. Research studies have been conducted to implement GS with different emphases in crop- and trait-specific applications, prediction models, design of training populations, and identifying factors influencing prediction accuracy. GS plays different roles in plant breeding such as turbocharging of gene banks, parental selection, and candidate selection at different stages of the breeding cycle. It can be enhanced by additional data types such as phenomics, transcriptomics, metabolomics, and enviromics. In light of the rapid development of artificial intelligence (AI), GS can be further improved by either upgrading the entire framework or individual components. Technological advances, research innovations, and emerging challenges in agriculture will continue to shape the role of GS in plant breeding.

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

## P236

### Getting priorities straight: Altering source-sink strength in *Sorghum bicolor* increases sugar production

(submitted by Lizeth Dominguez <lizethd2@illinois.edu>)
Full Author List: Dominguez, Lizeth[1]; Studer, Anthony J.[1]
[1] Department of Crop Sciences, University of Illinois Urbana-Champaign, Urbana, IL 61801, USA

Reducing greenhouse gas (GHG) emissions has been an important area of research to mitigate future climate change impacts. The use of Sustainable aviation fuel (SAF) reduces GHG emission by replacing non-renewable fuels. New technology can ferment feedstock sugars into SAF products that are directly implemented into current infrastructure. The Environmental Protection Agency reports that SAF consumption increased by 20 million gallons between 2021 and 2023, illustrating the increased demand for SAF feedstocks and the need for diversification of SAF feedstock sources. *Sorghum bicolor* is a potential feedstock for the SAF market however its sugar production levels are not yet sufficient for it to be viable feedstock. In spite of this, sorghum has multiple benefits as a feedstock such as high stress tolerance, high biomass, and the ability to grow on marginal land that could be leveraged if higher sugar levels could be achieved. Our work shows that the accumulation of sugar in the stem can be increased by removing the panicle as sink tissue. In a 3-year field trial two cytoplasmic male sterile (CMS) lines were grown side by side, with adjacent fertile lines. In each year, one CMS row was bagged (inhibiting seed set), and the other was open pollinated (seed producing). Among the six lines juiced, a range of performances were observed showing that sorghum can alter stem sink strength in a genotype dependent manner. Some lines showed significant increases in juice volume, while others showed no change, indicating underlying differences in source-sink programming. To further explore this, stem RNA-seq data has been collected on two lines with extreme juicing phenotypes to help dissect the underlying mechanism driving this phenomenon. Concluding our RNA-seq analysis, future work will target genes to help increase sorghum sugar production to support its viability as a feedstock for the SAF market.

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## P237

## High intensity phenotyping sites: Genetic regulation of phenotypic plasticity/stability.

(submitted by Huyu Liu <huyu@iastate.edu>)
Full Author List: Liu, Huyu[1]; Coffey, Lisa[1]; Turkus, Jonathan[2]; Corona-López, Lina[2 3]; Davis, Jensina[2]; Kusmec, Aaron[1 4]; Schnable, James C.[2]; Schnable, Patrick S.[1]
[1] Iowa State University, Ames, IA 50011, USA
[2] University of Nebraska-Lincoln, Lincoln, NE 68588, USA
[3] US Department of Agriculture Agricultural Research Service, Plant Science Research Unit, Raleigh, NC, 27607, USA
[4] Kansas State University, Manhattan, KS 66506, USA

In a world with a changing climate, the yield stability of crops has become a major concern. However, plant breeding has traditionally focused primarily on increasing yields per se rather than on yield stability across environments and years. To better understand the genetic control of phenotypic stability, crop traits and environmental data were collected over two years from 2-row plots of a inbred diversity panel grown in diverse environments from western Nebraska to eastern Iowa that span elevation and rainfall gradients. In 2022, three levels of N fertilizer were applied to two of the five locations. In 2023, two of four sites had three levels of N treatments. In total this project assayed crop performance in 17 distinct environments. Mutants of genes associated with variation in phenotypic stability by Kusmec et al. (Nat. Plants, 2017) were also evaluated across many of these environments. Multiple types of trait data (e.g., plant and ear height, a variety of ear traits) and environmental data were collected from these plots. The plasticity of nine traits (Ear Length, Ear Width, Hundred Kernel Mass, Kernel Mass Per Ear, Kernels Per Ear, Shelled Cob Mass, Shelled Cob Width, Ear Height and Plant Height) were analyzed via Finlay–Wilkinson regression. Association studies using SNPs and gene expression values as explanatory variables identified candidate genes for plasticity/stability for multiple traits.*This material is based upon work supported by the Agriculture and Food Research Initiative grant no. 2021-67021-35329/project accession no. IOWW-2021-07266, AI Institute for Resilient Agriculture (AIIRA); and grant no. 2020-68013-30934/project accession no. IOW05612, High Intensity Phenotyping Sites (HIPs) from the USDA National Institute of Food and Agriculture.*Kusmec A, S Srinivasan, D Nettleton, PS Schnable (2017) **Distinct genetic architectures for phenotype means and plasticities in *Zea mays***. **Nat Plants**, 3(9): 715-723. *doi:10.1038/s41477-017-0007-7*

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## P238

## Hybrid prediction in CHiDO: A friendly no-code genomic prediction tool for breeders

(submitted by Vitor Sagae <vitor.sagae@ufl.edu>)
Full Author List: Sagae, Vitor S[1 2]; Garcia-Abadillo, Julián[1]; González, Francisco[1]; Nascimento, Moysés[1 2]; Jarquin, Diego[1]
[1] University of Florida; Agronomy Department; Gainesville; Florida; United States 32608
[2] Federal University of Viçosa; Statistics Department; Viçosa; Minas Gerais; Brazil 36570-900

Genomic prediction (GP) techniques are powerful tools to employ in plant breeding programs. For example, in breeding programs seeking the development of hybrids, genomic prediction addresses the challenge of high number of potential combinations between inbred lines. Thus, fewer combinations are evaluated in fields and the collected information is used to train GP models for predicting the unobserved crosses, aiming to identify promising combinations to be further developed. Besides genomic information, accounting for genotype-by-environment interactions and including other data types such as UAV-based high throughput phenotyping, environmental factors, transcripts, metabolites, and others could increase predictive abilities of the models. However, implementing statistical models incorporating all these effects, and their interactions can be challenging due to the required programming and statistical skills. To overcome this limitation, we developed the platform called CHiDO, a friendly no-code interface designed to integrate multi-omics data to build, train and test models under different prediction scenarios of breeding programs. Currently, CHiDO can also build models focused on hybrid prediction based on the general and specific combining ability terms. The pipeline is divided into the following steps: uploading data, building models by simply dragging and dropping the main effects and their interactions, selecting cross-validation schemes, and fitting the models. The results, along with figures, can be easily viewed or downloaded. The metrics used to evaluate the models are the variance components, the within-environment and overall correlations, and the root mean square error (RMSE), between the predicted and observed values. CHiDO is an innovative tool for integrating multi-omics data in plant breeding and is continually being improved to incorporate additional tools to assist breeders in developing better genotypes.

Funding acknowledgement: Agronomy Department (UF/IFAS), UF/IFAS Florida Agricultural Experiment Station, CAPES, CNPq

## P239

## Impact of spontaneous haploid genome doubling on haploid induction in maize haploid inducers

(submitted by Vencke Gruening <venckegr@iastate.edu>)
Full Author List: Gruening, Vencke K[1]; Frei, Ursula K[1]; Chen, Yu-Ru[1]; Lubberstedt, Thomas[1]
[1] Department of Agronomy, Iowa State University, Ames, Iowa 50011, USA

Doubled haploid (DH) technology is a cornerstone of modern maize breeding, enabling the rapid development of homozygous inbred lines for hybrid production. This method significantly reduces the time required to develop inbred lines compared to traditional multi-generation self-pollination. A critical step in the DH process involves treating haploid seedlings with exogenous chemicals, such as colchicine, to induce genome doubling. However, this approach is costly, time-intensive, and involves handling toxic chemicals, necessitating careful precautions and additional labor. Spontaneous Haploid Genome Doubling (SHGD) offers a sustainable alternative by naturally inducing genome doubling, eliminating the need for chemical treatments, greenhouse space and the transplanting step. Integrating SHGD into DH inducer lines could reduce the cost and complexity of DH inducer line development. Developing inducer lines with increased haploid induction rates (HIR) will lead to higher rates of self-induced haploid plants, which will be male sterile and not shed pollen. Implementing SHGD into the DH inducer lines, will overcome this by restoring haploid male fertility (HMF). To evaluate whether HMF and HIR are independent traits, we developed a segregating haploid population from a cross between A427, which carries *qshgd1* (associated with SHGD), and BHI, which carries *mtl* (associated with HIR). The population was divided into two groups: one treated with colchicine and one untreated, and 500 plants in each group were genotyped. Preliminary results confirmed a previously reported segregation distortion against *mtl* and showed a higher rate of successful pollinations in plants carrying *qshgd1*. Moreover, preliminary results suggest no significant association between SHGD and HIR. These findings, though based on limited data, suggest potential for SHGD to facilitate the improvement of advanced HIR performance haploid inducers, since it would lead to more self-induced haploid plants with restored male fertility. Additional genotyping and phenotyping of larger populations next year will provide a better insight into the combined effects of these traits.

Funding acknowledgement: National Science Foundation (NSF), United States Department of Agriculture (USDA), R.F. Baker Center for Plant Breeding, Plant Sciences Institute, K.J. Frey Chair in Agronomy at Iowa State University

## P240

## Inactivation of a lysine-histidine transporter-1 gene confers southern leaf blight resistance in maize

(submitted by Qin Yang <qyang@nwafu.edu.cn>)
Full Author List: Wang, Yapeng[1]; Huang, Xiaojian[1]; Tian, Yuan[1]; Wang, Zhe[1]; Tai, Huanhuan[1]; Song, Weibin[2]; Balint-Kurti, Peter[3 4]; Yang, Qin[1]
[1] State Key Laboratory for Crop Stress Resistance and High-Efficiency Production, College of Agronomy, Northwest A&F University, Yangling 712100, P. R. China
[2] National Maize Improvement Center, Department of Plant Genetics and Breeding, China Agricultural University, Beijing 100193, P. R. China
[3] Department of Entomology and Plant Pathology, North Carolina State University, Raleigh, NC 27695, USA
[4] Plant Science Research Unit, USDA-ARS, Raleigh 27695, NC, USA

Southern leaf blight (SLB) is one of the most serious foliar diseases in maize worldwide. *qSLB$_{6.01}$* is a major quantitative trait locus conferring recessive resistance to SLB. Through map-based cloning, ethyl methanesulfonate mutagenesis, and CRISPR-Cas9 editing, we demonstrate that a lysine-histidine transporter-1 (*ZmLHT1*) gene at *qSLB$_{6.01}$* confers quantitative susceptibility to SLB. A 354 bp insertion in the *ZmLHT1* coding region in the resistant parental line NC292 creates a truncated protein, resulting in enhanced disease resistance to SLB. Targeted mutation of *ZmLHT1* leads to robust SLB resistance without affecting other important agronomic traits. *ZmLHT1* encodes a plasma membrane-localized broad-spectrum amino acid transporter. Transcriptome profiling reveals that genes involved in plant secondary cell wall biosynthesis were strongly induced in leaves of the *zmlht1* mutant after *Cochliobolus heterostrophus* infection, whilst cell redox homeostasis-related genes were highly expressed in wildtype plants. Moreover, we present evidence that ZmLHT1 reduces ROS deposition and inhibits secondary cell wall thickening during *C. heterostrophus* infection. Our findings may aid in disease resistance breeding through marker-assisted selection or genome editing while balancing growth-defense tradeoffs in maize.

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# P241

## Integrating proximal sensing modalities for enhanced prediction of agronomically important crop traits

(submitted by Erin Farmer <eef52@cornell.edu>)
Full Author List: Farmer, Erin E.[1]; Michael, Peter[2]; Gu, Zeqi[5]; Yan, Ruyu[2]; Wickes-Do, Liam[1]; Lepak, Nick[3]; Romay, Cinta[4]; Buckler, Edward S.[1 3 4]; Sun, Ying[6]; Robbins, Kelly[1]; Gage, Joseph L.[7]; Davis, Abe[2]; Gore, Michael A.[1]
[1] Plant Breeding and Genetics Section, School of Integrative Plant Science, Cornell University, Ithaca, New York, USA 14853
[2] Department of Computer Science, Cornell University, Ithaca, New York, USA 14853
[3] United States Department of Agriculture-Agricultural Research Service, Robert W. Holley Center for Agriculture and Health, Ithaca, New York, USA 14853
[4] Institute for Genomic Diversity, Cornell University, Ithaca, New York, USA 14853
[5] Cornell Tech, New York, New York, USA 10044
[6] Soil and Crop Sciences Section, School of Integrative Plant Science, Cornell University, Ithaca, NY, USA 14853
[7] Department of Crop and Soil Sciences, North Carolina State University, Raleigh, North Carolina, USA 27695

Proximal sensing technologies have enabled the collection of vast amounts of phenomic data for characterization of crop traits. However, data streams collected across sensors and platforms are predominately utilized separately due to heterogeneity in their structural, spatial, and spectral information. We aim to leverage advances in artificial intelligence to characterize plant form and health across temporal scales through integration of high-dimensional, multi-modal data. We collected over 75,000 multispectral images (MSIs) via unoccupied aerial vehicles (UAVs) and over 35,000 LiDAR scans via unoccupied ground vehicles (UGVs) for maize hybrids from the Genomes to Fields project in Aurora, NY from 2020 through 2024. Autoencoders, which are unsupervised, deep learning models, were implemented to extract latent features from each data type, producing reduced representations which contained biologically relevant information, with heritabilities up to ~0.9. MSI and LiDAR latent features were integrated at the plot-level across all time points, and Bayesian regression was used to predict manually measured phenotypes. These predictions were compared against 49 vegetation indices (VIs) which had accuracies ranging from ~0.26 for stalk lodging to ~0.85 for days to anthesis. Integrated latent features outperformed VIs for all phenotypes except for flowering time traits and grain moisture, ranging from a decrease in accuracy of ~8.8% for grain moisture to an increase of ~19.0% for ear height. Across all traits, integrated latent features increased the prediction accuracy by an average of ~4.6%. Notably, integrated latent features also provided a ~5.1% and ~20.8% increase in accuracy over the individual use of MSI and LiDAR latent features, respectively. We show that latent phenotyping circumvents manual curation of features and allows integration across modalities, improving characterization of key crop traits and further facilitating the deployment of proximal sensors for use in precision agriculture and breeding programs.

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

## P242

## Integration of doubled haploid technology with marker-assisted techniques for fixing major genes to develop specialty corn

(submitted by Tae-Chun Park <tcpark@iastate.edu>)
Full Author List: Park, Tae-Chun[1]; Frei, Ursula[1]; Lübberstedt, Thomas[1]; Scott, Paul[2]
[1] Department of Agronomy, Iowa State University, Ames, Iowa, 50011
[2] USDA-ARS-CICGRU, Ames, Iowa, 50011

The application of doubled haploid (DH) technology in maize breeding has gained significant attention due to its efficiency. First, it enables the production of 100% homozygous lines, reducing time required for inbreeding from 8-9 generations to two generations. This is achieved through genome doubling from haploids to diploids. In addition to reducing the time required to produce inbred lines, DH technology also offers a benefit in fixing multiple desired genes or QTLs. In backcross breeding programs, DH technology significantly reduces the population size needed to fix target genes compared to conventional breeding population, even though it does not accelerate the overall BC process. In the haploid phase, a larger proportion of progeny contain the desired gene combination than in the diploid phase because the heterozygous class is not present. Notably, if the desired trait is present at the haploid stage, the trait will be fixed in the DH lines through the haploid genome doubling process, regardless of whether the trait is dominant or recessive. The benefits of DHs, therefore, become more pronounced as the number of target genes increases, making it particularly effective in efficiently fixing multiple target genes in BC programs. For example, the minimal backcross population size required to find an individual with the desired combination of alleles of four recessive genes at the 99% probability level in the haploid stage is 71.36 while 1176.62 are required in the diploid stage. Here, we aim to develop two types of specialty corn by integrating marker-assisted backcrossing (MABC) and DH technology to combine four major genes. These two types of specialty corn are developed to meet specific market demands, by combining four target genes including waxy 1, yellow 1, spontaneous haploid genome doubling (SHGD), and Gametophyte factor 1 (Ga1-s) or opaque-2, brown-midrib 3, SHGD, and Gametophyte factor 1 (Ga1-s).

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

## P243

## Investigating the role of a group XIIa LRR receptor-like kinase in maize resistance against *Xanthomonas vasicola* pv. *vasculorum*

(submitted by Shuya Wang <shuyaw2@illinois.edu>)
Full Author List: Wang, Shuya[1]; Geedey, Aislinn[1]; Mullens, Alexander[1]; Jamann, Tiffany[1]
[1] Department of Crop Science; University of Illinois at Urbana- Champaign; Urbana, Illinois, 61802

Group XIIa receptor-like kinases (RLKs) with leucine-rich repeat (LRR) domains play crucial roles in plant defense by recognizing pathogen-associated molecular patterns (PAMPs) and initiating immune responses. In this study, we investigated the role of a Group XIIa LRR-RLK in maize resistance to *Xanthomonas vasicola* pv. *vasculorum* (*Xvv*), the causal agent of bacterial leaf streak disease. We utilized near-isogenic lines (NILs) with putative introgressions at the target locus from donors that lack a functional version of the target LRR-RLK and UniformMu maize lines carrying transposon insertions in the target LRR-RLK to perform resistance assays. Plants were evaluated for vascular and non-vascular disease after tissue-specific inoculations. Genotyping confirmed the presence of introgressions or transposon insertions. Together, the disease screenings of the NILs and UniformMu lines indicate that the candidate LRR-RLK gene might mediate resistance to *Xvv*, particularly against non-vascular infections, while offering limited protection against vascular infections. This study identifies a potentially novel genomic region associated with *Xvv* resistance, as no quantitative trait loci (QTL) associated with *Xvv* resistance have been reported in this area to date. These findings offer insights into the function of this receptor-like kinase in disease resistance in maize.

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## P244 @phenomics_oz

### Leveraging UAV-based hyperspectral imaging and machine learning models for prediction of SPAD in maize

(submitted by Manoj Subedi <msubedi@udel.edu>)
Full Author List: Subedi, Manoj[1]; Kim, Jeonghwa[1]; Bao, Yin[1 2]; Mu, Qi[1]
[1] Department of Plant and Soil Sciences, University of Delaware, Newark, Delaware, USA, 19711
[2] Department of Mechanical Engineering, University of Delaware, Newark, Delaware, USA, 19711

Soil plant analysis development (SPAD) is a widely used indicator of the relative chlorophyll content of a plant. Measurement of phenotypic traits like SPAD is a labor- and time-intensive task. The combination of hyperspectral imaging (HSI) and machine learning (ML) hold tremendous potential for high-throughput phenotyping tasks. This study assessed ML models for prediction of SPAD using features derived from unmanned aerial vehicles (UAV)-based hyperspectral imagery for a maize screening trial for water-logging stress in 2024. Using hyperspectral bands, 81 vegetation indices were extracted to train and evaluate four ML models: K-nearest neighbors, support vector regression, gradient boosting regression, and multi-layer perceptron (MLP). To assess the utility of full-spectrum reflectance data, a one-dimensional convolution neural network was trained using the mean and standard deviation of 158 reflectance values as input features. In-season SPAD data was measured using a hand-held spectrometer at 12 days, 26 days and 56 days after application of the water-logging treatment. All models were tested for their accuracy to predict SPAD based on standard metrics with ten-fold cross-validation. Prediction accuracy for the MLP model marginally outperformed rest of the models. Feature selection and feature importance analyses will be performed to identify key vegetation indices as HSI-derived indicators for SPAD and water-logging stress. This study revealed the feasibility of using UAV-based hyperspectral imaging technique combined with ML models to assess the health and performance of large number of genotypes in a high-throughput manner.

## P245

### Maize genomic prediction: Integrating genomics and transcriptomics for trait analysis

(submitted by Ally Schumacher <schum193@msu.edu>)
Full Author List: Schumacher, Ally M.[1]; Sitar, Sidney[2]; Gomez Cano, Lina[3]; Brottlund, Donielle[2]; Newton, Linsey[2]; Turkus, Jon[4 5 6]; Schnable, James C.[4 5 6]; Grotewold, Erich[3]; Thompson, Addie[2]
[1] Department of Plant Biology; Michigan State University, East Lansing, MI 48824, USA
[2] Department of Plant, Soil and Microbial Sciences, Michigan State University, East Lansing, MI, 48824, USA
[3] Department of Biochemistry and Molecular Biology, Michigan State University , East Lansing, MI, 48824, USA
[4] Quantitative Life Sciences Initiative, University of Nebraska-Lincoln, Lincoln, NE, 68588, USA
[5] Center for Plant Science Innovation, University of Nebraska-Lincoln, Lincoln, NE, 68588, USA
[6] Department of Agronomy and Horticulture, University of Nebraska-Lincoln, Lincoln, NE, 68588, USA

Tar spot disease in maize, caused by the fungal pathogen *Phyllachora maydis*, can reduce yield by up to 40% depending on infection severity. Polyphenolic compounds have been preliminarily linked to tar spot resistance, suggesting a relationship between compound accumulation and plant disease response. Pigmentation traits, such as anther and silk coloration, are influenced by polyphenolic compounds and may be connected to shared genetic pathways and regulatory networks underlying both aesthetic and disease resistance traits. Genome-wide association studies (GWAS) are being conducted to investigate these traits, focusing on identifying shared pathways and regulatory mechanisms that link phenolic compound accumulation, pigmentation, and tar spot resistance. Significant single nucleotide polymorphisms (SNPs) identified through GWAS will be incorporated into genomic prediction models to evaluate their utility in predicting phenotypic variation in pigmentation traits and phenolic accumulation. To complement this, transcriptome-wide association studies (TWAS) and whole genome comparative network analyses (WGCNA) are being conducted to identify genetic loci and expression networks associated with tar spot severity. These analyses leverage a Wisconsin Diversity Panel evaluated over multiple years and locations to study regulatory networks influencing phenolic compound accumulation in relation to plant susceptibility to tar spot disease. By combining GWAS-identified loci with co-expression patterns from WGCNA and trait-associated transcripts from TWAS, this study aims to identify candidate genes associated with both pigmentation and phenolic traits. This integrative approach is expected to enhance the discovery of functional variants and improve genomic prediction accuracy, particularly for breeding applications targeting tar spot disease resistance.

# P246

## Maize hybrid stability and weather variables associated with ear rot resistance and mycotoxin accumulation

(submitted by Sarah Lipps <slipps@illinois.edu>)
Full Author List: Lipps, Sarah[1]; Hasty, Charlette[2]; Butts-Wilmsmeyer, Carolyn[2 3]; Bohn, Martin[1]; Jamann, Tiffany[1]
[1] University of Illinois, Urbana, IL Department of Crop Sciences
[2] Southern Illinois University, Edwardsville, IL Department of Biological Sciences
[3] Southern Illinois University, Edwardsville, IL Center for Predictive Analytics

Maize is under perpetual threat from pathogens. Ear rots are particularly threatening, as fungal colonization decreases grain quality. Common ear rots are Gibberella ear rot caused by *Fusarium graminearum,* Fusarium ear rot caused by *Fusarium verticillioides,* and Aspergillus ear rot caused by *Aspergillus flavus.* These fungi produce mycotoxins during colonization which are harmful when consumed. Our hypothesis is that distinct environmental covariates (ECs) and their timing are drivers for ear rot and mycotoxin incidence. Our objectives were to 1) examine the geographic distribution of multiple ear rots, 2) identify key ECs that are drivers for incidence of individual ear rots and mycotoxin accumulation, and 3) quantify the relationship between ECs and ear rot incidence and mycotoxin presence. We developed a hybrid population (n=108) by selecting and intermating parents categorized as resistant, susceptible, or moderately susceptible to Gibberella ear rot. Through collaborations with Beck's Hybrids and the Genomes-to-Fields Initiative, we screened the entire hybrid population in four environments between 2023 and 2024 and a subset representative of potential ear rot resistance in 18 additional environments between 2023 and 2024. Fusarium ear rot was prevalent across all environments, and Gibberella ear rot was most prevalent in northern environments. To further understand the role of the environment on ear rot development, we generated 40 ECs within non-overlapping 15-day windows starting 60 days after silking, working backward until 30 days before silking in each environment. We compared mixed models that include each EC as a fixed covariate to a null model with no EC to identify the most significant ECs in each window associated with each ear rot and mycotoxin. We conducted a correlation analysis to identify positive and negative relationships between different ECs and ear rot incidence. Multi-environment trials are useful for exploring the role of weather in ear rot resistance. Future directions include identifying the best and worst performing hybrids in each environment and across the entire dataset.

Funding acknowledgement: United States Department of Agriculture (USDA)

## P247
## Managing plot orientation and canopy architecture to improve overall maize grain yield

(submitted by Greg Schoenbaum <gregorys@iastate.edu>)
Full Author List: Schoenbaum, Gregory R.[1]; Alladassi, Boris M.E.[1]; Li, Dongdong[1]; Li, Xianran[2]; Yu, Jianming[1]
[1] Department of Agronomy, Iowa State University, Ames, IA 50011 USA
[2] USDA-ARS, Wheat Health, Genetics, and Quality Research, Pullman, WA 99164 USA

It is well understood that the combined effects of genetics, environment and management practices ("G x E x M") are major determinants of maize plant growth, health and overall yield. It is also understood that maize canopy architecture is an important determinant of light interception, which has a direct effect on the amount of energy available for photosynthesis and, ultimately, grain production. One historically successful manipulation of management practices, for example, has been increasing the number of plants on a given area of land, by sowing plants more closely together within a row or planting fields to narrower rows (or both). This was done under the premise that more plants mean more ears and, therefore, more grain. Unfortunately, in recent years, gains from this method have diminished, and population densities that are too high can create conditions sufficiently stressful to result in yields lower than expected. In an endeavor to identify additional methods for improving yield, in consideration of the standing debate that planting direction of row crops can impact productivity, we investigated whether altering plot orientation can be used to increase the interception of incoming sunlight and, as a result, total grain production. To accomplish this, we used a split-plot experimental design comprised of twenty maize testcross hybrids with distinct canopy architectures (flat, upright, or dynamic) at two locations in Iowa, USA. The experiment consisted of two replications of two-row plots planted either parallel or perpendicular to the general path of incoming solar radiation. Data were collected for population density (plants per acre), canopy light interception at various growth stages, grain moisture and grain yield. Identifying combinations of canopy architectures and plot orientations that maximize light interception could provide producers with an effective means for increasing overall grain yields, using resources that are readily available and easy to implement.

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

## P248 @olamide_olamz
## Mining genetic resistance to Goss's wilt of maize

(submitted by Olamide Adesina <oadesina@ksu.edu>)
Full Author List: Adesina, Olamide[1]; White, Frank[2]; Liu, Sanzhen[1]
[1] Kansas State University; Department of Plant Pathology; Manhattan, KS, USA 66506
[2] University of Florida; Department of Plant Pathology; Gainesville, FL,USA 32611

Goss's bacterial wilt and leaf blight (GW), caused by the Gram-positive actinobacterium *Clavibacter nebraskensis* (Cn), is the most significant bacterial disease affecting maize in the US and Canada. Genetic resistance remains the most effective control strategies. The genetic mechanisms underlying resistance to Cn, and more broadly to Gram-positive actinobacteria, remain poorly understood. Our group employs map-based cloning to dissect GW resistance. In addition, we screened the following genetic mutants for GW resistance: 1) Lesion mimic mutants, 2) mutants of Cn-responsive genes involved in phytohormone pathways, and 3) CRISPR-based knockout mutants of the maize homologs of the *Wall Are Thin1* (*WAT1*) gene that has been recently demonstrated to enhance resistance to *Clavibacter* bacteria in tomato. We have collected dozens of mutants, and the results from the phenotypic screening will be presented. We anticipate that this approach will complement map-based cloning methods, enabling the identification of genetic elements conferring GW resistance.

Gene / Gene Models described: *wat1*; Zm00001eb039040
Funding acknowledgement: Corteva Inc.

## P249

# Multi-environmental transcriptome-wide association study reveals the extent of genotype-by-environment interactions for flowering time in maize.

(submitted by Ty Thomas <tsthoma2@ncsu.edu>)
Full Author List: Thomas, Ty S.[1 2]; Torres-Rodríguez, J. Vladimir[3 4 5]; Turkus, Jon[3 4 5]; Sekhon, Rajandeep S.[6]; Wallace, Jason G.[7 8]; Washburn, Jacob D.[9]; Thompson, Addie M.[10 11]; Schnable, James C.[3 4 5]; Gage, Joseph L.[1 2]
[1] Department of Crop and Soil Sciences, North Carolina State University, Raleigh, NC 27695
[2] NC Plant Sciences Initiative, North Carolina State University, Raleigh, NC, 27606
[3] Quantitative Life Sciences Initiative, University of Nebraska-Lincoln, Lincoln, NE, 68588, USA
[4] Center for Plant Science Innovation, University of Nebraska-Lincoln, Lincoln, NE, 68588, USA
[5] Department of Agronomy and Horticulture, University of Nebraska-Lincoln, Lincoln, NE, 68588, USA
[6] Department of Genetics and Biochemistry, Clemson University, Clemson, SC 29634, USA
[7] Institute of Plant Breeding, Genetics, and Genomics, University of Georgia, Athens, GA, USA
[8] Department of Crop and Soil Science, University of Georgia, Athens, GA, USA
[9] USDA-ARS, MWA-PGRU, 302-A Curtis Hall, University of Missouri, Columbia, MO 65211, USA
[10] Department of Plant, Soil & Microbial Sciences, Michigan State University, East Lansing, MI 48824, USA
[11] Plant Resilience Institute, Michigan State University, East Lansing, MI 48824, USA

Multi-environmental transcriptome-wide association studies (TWAS) enable the identification of genes associated with flowering time both within, and across environments. Flowering time in maize is a key phenotypic trait that correlates to adaptability in tropical or temperate climates. As climates change, genetic components of flowering time need to be better characterized to ensure the fitness of future maize hybrids. To begin this characterisation, maize data were collected by the Genomes to Fields project resulting in a unique RNA sequencing dataset including 1,550 individual samples distributed across 6 environments with 498 different maize hybrid genotypes represented. We tested for association between gene expression and flowering time either considering individual environments separately or multiple environment data jointly. The TWAS for individual environments resulted in environment-specific genes associated with flowering time. The multi-environmental TWAS identified genes associated with flowering time regardless of the environment in which the plant was grown. From these results, we identified a genotype by environment interaction (GxE) occuring in gene expression levels and evaluate biological methods of regulating flowering time in differing environments. Further investigation into the genes associated with flowering time will result in a deeper knowledge of GxE and the ability to better select lines for specific environments and future climates.

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

## P250 @vla_torres

### Multi-species transcriptome-wide association studies identify additional genes controlling flowering

(submitted by Vladimri Torres-Rodriguez <jtorres-rodriguez2@unl.edu>)
Full Author List: Torres-Rodríguez, J. Vladimir[1 2 3]; You, Chong[4]; Turkus, Jon[1 2 3]; Qiu, Yumou[4 5]; Schnable, James C.[1 2 3]
[1] Quantitative Life Sciences Initiative, University of Nebraska-Lincoln, Lincoln, NE, 68588 USA
[2] Center for Plant Science Innovation, University of Nebraska-Lincoln, Lincoln, NE, 68588 USA
[3] Department of Agronomy and Horticulture, University of Nebraska-Lincoln, Lincoln, NE, 68588 USA
[4] School of Mathematical Sciences and Center for Statistical Science, Peking University, Beijing, People's Republic of China
[5] Department of Statistics, Iowa State University, Ames, IA 50010, USA

In principle, integrating quantitative data across related species should increase statistical power for identifying functional variation in genes of interest. However, equivalent genetic markers will typically not be present in multi-species analyses making this approach intractable for methods such as genome-wide association studies. In contrast, in transcriptome-wide association studies where gene expression rather than genetic markers are treated as explanatory variables, it is frequently possible to define orthologous transcripts between related species, making multispecies association tests more feasible. Here we develop and evaluate a new conditional method for transcriptome-wide association which enables joint testing across either paralogous gene pairs in a single species or orthologous gene pairs in related species. We evaluated this method using gene expression and trait data collected from 749 maize genotypes, searching for genes linked to anthesis in maize, either considering each gene independently or jointly evaluating pairs of homologous genes resulting from the maize whole genome duplication and we found 25 and 46 associated genes, respectively. We extended our analysis using gene expression and flowering time data from 811 sorghum genotypes. This allowed us to test for links between gene expression and flowering time data for 20,564 maize-sorghum syntenic orthologous gene pairs. Multispecies conditional TWAS for flowering time identified 74 gene pairs whose transcript abundance was correlated with flowering time variation which were not identified in single species analyses. This set of genes was significantly enriched among gene targets by selection during the adaptation of tropical maize to temperate latitudes -- which required significant changes in flowering time regulation -- and also included two gene pairs (SPL13/Zm00001eb105640/Sobic.002G312200) and (SPL29/Zm00001eb322280/Sobic.002G312200) which were recently confirmed to play a role in determining flowering time via Cas9 genome editing in an independent study.

Gene / Gene Models described: *zmspl13*, *zmspl29*; Zm00001eb105640, Zm00001eb322280
Funding acknowledgement: United States Department of Agriculture (USDA), Department of Energy (DOE), Department of Energy Advanced Research Projects Agency-Energy (ARPA-E)

## P251 @prashant87_

### Multiple conserved loci underlie plant water use efficiency in Sorghum and Setaria

(submitted by Prashant Bhandari <prash.danforth@gmail.com>)
Full Author List: Bhandari, Prashant[1]; Luebbert, Collin[1]; Ozersky, Philip[1]; Barrett, Jennifer[1]; Ziegler, Greg[1]; Mockler, Todd[1]; Baxter, Ivan[1]
[1] Donald Danforth Plant Science Center

Improving water use efficiency (WUE) is crucial for economic viability and agricultural sustainability of bioenergy feedstocks. However, water use efficiency at the plant level (WUE.plant) has remained largely unexplored. Researchers have primarily focused on leaf-level WUE, which doesn't scale to WUE.plant. To address this critical knowledge gap, we quantified biomass through imaging and measured water use of over 11,000 C4 plants from Sorghum and Setaria diversity panels at daily intervals under well-watered and water-limited conditions in 10 experiments and performed over 890 genome-wide association (GWA) mappings for WUE.plant and correlated traits. Furthermore, we integrated the GWA summary statistics using a Bayesian meta-analysis, and leveraged synteny to reduce false positives. Our study identified numerous conserved loci significantly associated with WUE.plant and correlated traits. These findings enable targeted improvement of Sorghum for WUE.plant, with potential implications for sustainable bioenergy feedstock production in C4 grasses.

Funding acknowledgement: Department of Energy (DOE)

## P252

## Natural alleles of the gene lhcb6 shape photosynthesis and key agronomic traits in maize (Zea mays L.) landraces

(submitted by Lukas Würstl <l.wuerstl@tum.de>)
Full Author List: Würstl, Lukas[1]; Urzinger, Sebastian[1]; Avramova, Viktoriya[1]; Urbany, Claude[2]; Scheuermann, Daniela[2]; Presterl, Thomas[2]; Mayer, Manfred[1]; Haberer, Georg[3]; Ordas, Bernardo[4]; Brajkovic, Sarah[5]; Ouzonova, Milena[2]; Schön, Chris-Carolin[1]
[1] Plant Breeding, TUM School of Life Sciences, Technical University of Munich, 85354 Freising, Germany
[2] Maize Breeding, KWS SAAT SE & Co. KGaA, 37574 Einbeck, Germany
[3] Plant Genome and Systems Biology, Helmholtz Center Munich, 85764 Neuherberg, Germany
[4] Spanish National Research Council (CSIC), Misión Biológica de Galicia, Apartado 28, 36080 Pontevedra (Spain)
[5] Chair of Proteomics and Bioanalytics, Technical University of Munich (TUM), 85354 Freising, Germany

Utilization of native genetic diversity can expand the genetic basis of traits exhibiting limited variation in breeding material. In a genome-wide association study in a DH library derived from three maize landraces we identified quantitative trait loci (QTL) associated with Fv/Fm (maximum potential quantum yield of photosystem II) and key agronomic traits (EME: emergence; PH: plant height; EV: early vigor; MF: male flowering). A QTL on chromosome 10 exhibited stable effects across all traits and was therefore selected for further investigation. Field and growth chamber experiments facilitated the fine-mapping of the QTL to a 65 kb region containing seven gene models. Our research uncovered the presence of a hAT transposon insertion in the promoter region of one of these genes, light harvesting chlorophyll a/b binding protein 6 (lhcb6). We demonstrate that this insertion reduces mRNA and protein levels, substantiating its role as the causal factor underlying the QTL. LHCB6 is a component of the PSII antenna light-harvesting complex and affects variation in the photosynthetic traits Fv/Fm and non-photochemical quenching (NPQ). We show in near isogenic lines (NILs) that the insertion in *lhcb6* is also associated with the traits EME, PH, EV, MF, assimilation rate and plant biomass. Our work provides novel insights into the function of *lhcb6* in the LHCII complex and demonstrates the value of natural variation for improving elite germplasm.

Funding acknowledgement: Federal Ministry of Education and Research (BMBF)

## P253

## Natural and orthogonal interaction models for gene-environment and gene-gene interactions

(submitted by Aaron Kusmec <amkusmec@ksu.edu>)
Full Author List: Kusmec, Aaron[1]
[1] Kansas State University; Manhattan, KS, USA 66506

Phenotypes develop over time in complex environmental and genic contexts. These factors interact to form phenotypes; however, as the number of interactants increases, the conceptual and analytical challenge also increases. In the case of gene-environment interactions (GEI), a two-step solution is commonly adopted where the genome is scanned for loci significantly associated with a trait of interest followed by a second scan for associations between the step-one loci and possible environmental covariates. Alternatively, a norm of reaction between the trait of interest and one or more environmental covariates is estimated followed by genome scans on the reaction norm parameters. These two approaches are mathematically equivalent but make less efficient use of the available information than a single-step approach would. The natural and orthogonal interactions (NOIA) model was developed to model epistasis (gene-gene interaction; GGI) in arbitrary populations using an orthogonal coding of the genetic effects such that epistatic effects of arbitrary order are efficiently computed when adding loci to the model and effects are consistently estimated during model selection. This work extends the NOIA framework by constructing an orthogonal coding of polynomial functions of one or more environmental covariates of arbitrary order. Combined with the orthogonal genetic coding of the original NOIA framework, this extension provides a single-step strategy for identifying GEI, GGI, and gene-gene-environment interactions (GGEI) with a parsimonious model selection procedure. The utility of the extended NOIA model is demonstrated on flowering time and plant height collected in multiple environments on a sorghum RIL population for genetic mapping and phenotypic prediction.

## P254

### Non-destructive visualization of alpha-zein expression and grain protein in maize using the FLOURY2-RFP reporter transgene

(submitted by Catherine Li <chli6@illinois.edu>)
Full Author List: Li, Catherine[1]; Heller, Nicholas[2]; Arp, Jennifer[1 3]; Moose, Steve[1]
[1] Department of Crop Sciences, University of Illinois Urbana-Champaign, Urbana, IL, USA, 61801
[2] Department of Agriculture, Illinois State University, Normal, IL, USA, 61761
[3] Bayer Crop Science, Chesterfield, MO, USA, 63017

The FLOURY2-RFP reporter gene, initially developed by Anne Sylvester and David Jackson labs, enables rapid, non-destructive visualization of alpha-zein accumulation in individual maize kernels under white light. This feature is due to the high expression level of the FLOURY2 gene, the stability of zein proteins, and the use of monomeric RFP, which emits fluorescence without the need for multimerization. We have previously shown that the single-locus FLOURY2-RFP reporter effectively tracks the expression profile of the entire 22-kDa alpha-zein subfamily, and even total grain protein concentration. We have measured the pink color intensity of the FLOURY2-RFP reporter transgene in various maize populations by extraction of spectral features from kernel images using FIJI software. These same kernels were also measured with near-infrared reflectance to estimate total grain protein concentration. RFP intensity was strongly correlated with total grain protein concentration in populations derived from the Illinois Long-Term Selection experiment, where phenotypic variation for zein content is genetically controlled. However, unexplained variance remains which will offer insights into the genetic control of seed protein accumulation that is distinct from the well-studied zeins. Additionally, the FLOURY2-RFP reporter has demonstrated sensitivity to nitrogen fertilizer application. To further explore this, both historical and modern hybrids were grown under nitrogen-limited and nitrogen-sufficient conditions and cross-pollinated with plants carrying the FLOURY2-RFP reporter gene. Analysis of FLOURY2-RFP intensity and grain protein concentrations in these open-pollinated hybrids reinforced that the genetics of the maternal source plant determines both traits. Our findings highlight the FLOURY2-RFP reporter gene as a valuable tool for investigating grain protein composition in maize.

Funding acknowledgement: Illinois Corn

## P255

### Oaxacan green dent maize is not from Oaxaca

(submitted by James Holland <Jim.Holland@usda.gov>)
Full Author List: Holland, James B.[1 2]; Willcox, Martha C.[3]; Samayoa-Lopez, Fernando[2]; Woore, Matthew S.[2]; Salazar-Vidal, Miriam N.[4]; Tracy, William F.[5]
[1] USDA-ARS Plant Science Research Unit, Raleigh, NC, USA 27606
[2] Department of Crop and Soil Sciences, North Carolina State University, Raleigh, NC, USA 27606
[3] Winston-Salem, NC, USA 27104
[4] Division of Plant Sciences and Technology, University of Missouri, Columbia, MO 65211, USA
[5] Department of Plant and Agroecosystem Sciences, University of Wisconsin-Madison, Madison, WI, USA 53711

“Oaxacan Green Dent” is a maize cultivar marketed as an introduced Mexican heritage variety adapted to the higher latitudes of the USA. Its adaptation and appearance contradict an origin in Oaxaca, Mexico, however, and no indigenous cultivars in Oaxaca are known to have the unique kernel colors of Oaxacan Green Dent. We compared phenotypes and genotypes of Oaxacan Green Dent sampled from three different sources along with several Corn Belt cultivars and 15 cultivars collected from a wide range of geography, altitude, and cultural groups in Oaxaca. Multivariate analysis of 13 phenotypic traits measured in a field experiment suggested that Oaxacan Green Dent is more closely related to Corn Belt Dents than to Oaxacan cultivars. Genomic analysis from DNA sequencing demonstrated unambiguously that Oaxacan Green Dents are even more distantly related to Oaxacan cultivars than typical USA Corn Belt Dent cultivars are. Phenotypic, genetic, and historical data indicate that Oaxacan Green Dent is almost certainly directly derived from Ernest Strubbe’s Green Dent cultivar, which he developed in Minnesota from crosses between a Corn Belt Dent cultivar and an intensely pigmented popcorn variety, with no contribution from Oaxacan cultivars.

Funding acknowledgement: United States Department of Agriculture (USDA)

## P256

### Optimizing maize growth in growth chambers

(submitted by Shreejana KC <sreezaa20@gmail.com>)
Full Author List: K C, Shreejana[1]; Subedi, Manoj[1]; Kim, Jeonghwa[1]; Meng, Qingwu[1]; Mu, Qi[1]
[1] University of Delaware; 531 S College Ave; Newark, DE, USA 19716

Controlled environments precisely regulate key environmental factors influencing plant growth, enabling efficient study of genetic-environment interactions and promoting sustainable agriculture. Growth chambers are widely accessible and versatile controlled environments, serving as ideal resources for plant-based experiments. Maize, a resilient staple C4 crop, holds potential as a calorie-dense resource. Understanding maize growth in controlled environments is critical to optimizing its performance in both traditional and non-traditional settings, such as vertical farming and space-based agriculture. However, maize's high light and spatial demands hinder its indoor cultivation. To address these challenges, we used a dwarf mutant genotype and retrofitted growth chambers with energy-efficient LED lighting to support maize growth from germination to maturity. Two light intensities averaging around 1000 µmol/m²/s and 500 µmol/m²/s throughout the growing period were tested. A completely randomized design was applied to the dwarf mutant and a standard height inbred line B73. Phenotypic and physiological traits, including canopy height, stalk diameter, relative chlorophyll content (measured by Soil Plant Analysis Development, SPAD), and photosynthetic efficiency (represented by Phi2) were measured throughout the growing season. Results demonstrate that integrating LED lighting in growth chambers supports maize development from germination to maturity for both genotypes, though growth patterns differed by light intensities. This work provides a framework for standardized indoor maize cultivation in growth chamber systems and enables future research on gene-environment interactions, vertical farming, and space-based agriculture.

Funding acknowledgement: NASA EPSCoR

## P257 @XiangZhaocheng

### Parametrization and quantification of maize leaf morphology for phenotyping and quantitative genetics

(submitted by Zhaocheng Xiang <zxiang2@unl.edu>)
Full Author List: Xiang, Zhaocheng[1]; Ge, Yufeng[1]
[1] Department of Biological Systems Engineering, University of Nebraska-Lincoln, Lincoln, NE 68583, USA

Current maize morphological traits for phenotyping and quantitative genetics are coarse and bulky, such as plant height, leaf length, leaf angle, etc. Precise and comprehensive description of the morphological traits at the sub-organ level is still an open question due to the geometric complexity. To fill the emptiness in maize leaf, we proposed a modeling method to demonstrate morphological traits on maize leaf mathematically and quantitatively by utilizing parametric curves. A maize leaf is decomposed into three elements: midrib, cross-section and blade contour. Those elements are fitted by hyperbolic spiral, tractrix curve and Sanderson equation, respectively. A realistic maize leaf model can be generated by translation, rotation and scaling of the three elements in 3D space. Consequently, leaf morphological traits are expressed and quantified by the parameters that control the shapes of the curves. The modeling method is bidirectional which builds a bridge between the real world and virtual reality. Compared with other maize models, our method allows complete and numerical description of subtle morphological features, including curvature and twisting of the midrib, undulation extent, blade included angle, etc. The parameterization and quantification of these complex morphologies enable maize phenotyping at the leaf level, facilitating advancements in quantitative genetics research related to maize leaf traits.

Funding acknowledgement: United States Department of Agriculture (USDA)

# P258

## Patterns of selection for adaptation to spatial and temporal fluctuating nitrogen availability in maize

(submitted by Gen Xu <gxu6@unl.edu>)
Full Author List: Xu, Gen[1 2]; Lyu, Jing[3]; Wang, Zhen[1 2]; Ulutas, Musa[1 2]; Obata, Toshihiro[2 4]; Ge, Yufeng[2 5]; Michaels, Scott D.[3]; Liu, Sanzhen[6]; Schnable, James C.[1 2]; Yang, Jinliang[1 2]
[1] Department of Agronomy and Horticulture, University of Nebraska-Lincoln, Lincoln, NE 68583, USA.
[2] Center for Plant Science Innovation, University of Nebraska-Lincoln, Lincoln, NE 68583, USA.
[3] Department of Biology, Indiana University, Bloomington, IN 47405, USA.
[4] Department of Biochemistry, University of Nebraska-Lincoln, Lincoln, NE 68588, USA.
[5] Department of Biological Systems Engineering, University of Nebraska-Lincoln, Lincoln, NE 68583, USA.
[6] Department of Plant Pathology, Kansas State University, Manhattan, KS 66506, USA.

Understanding the patterns of selection during plant evolution and recent crop improvement processes is a central topic in plant genetics and is critical for breeding climate-resilient crops. Nitrogen (N), as an essential macronutrient for plant growth and development, exhibits spatial and temporal variability in natural ecological and agriculture production systems worldwide and is a key factor affecting plant adaptation and crop improvement. In this study, we analyzed geographically adaptive tropical maize materials and historical maize inbred lines developed under varying N regimes. The introgression of tropical materials into temperate genetic backgrounds reduced inbred phenotypic performance, while a number of individual beneficial alleles contribute to N-resiliency. Population genomics analyses identified $n$ = 49 genetic loci under balancing selection in elite tropical materials that have undergone recent positive selection during recent breeding, some of which contain genes involved in N metabolism and N stress signaling pathways. Among the potential N-related positive selective sweeps, a cluster of three glutamate receptor-like *ZmGLR3.4* genes is associated with several morphological, physiological, and metabolite traits collected under different N conditions. A series of *Zmglr3.4* mutants under different genetic backgrounds validated the N-resilient phenotypic and transcriptomic responses. These results revealed the N resilient effects of the historical alleles at the *ZmGLR3.4* locus and shed light on potential targets for enhancing N use efficiency.

Funding acknowledgement: United States Department of Agriculture (USDA)

## P259

## Phenome-to-genome insights for evaluating root system architecture in field studies of maize

(submitted by Kirsten Hein <khein@danforthcenter.org>)
Full Author List: Hein, Kirsten M[1 2]; Liu, Alexander E[2]; Mullen, Jack L[1]; Shao, Mon-Ray[2 3]; Topp, Christopher N[2]; McKay, John K[1]
[1] Colorado State University; 307 University Street; Fort Collins, Colorado, USA 80525
[2] Donald Danforth Plant Science Center; 975 North Warson Road; Saint Louis, Missouri, USA 63132
[3] Dümmen Orange; Oudecampsweg 35C; De Lier, NLD 2678

Understanding the genetic basis of root system architecture (RSA) in crops requires innovative approaches that enable both high-throughput and precise phenotyping under field conditions. In this study, we evaluated multiple phenotyping and analytical frameworks for quantifying RSA in mature, field-grown maize (*Zea mays* L.) across three field experiments. Using forward and reverse genetic approaches, we analyzed over 1,700 maize root crowns, including a diversity panel, a biparental mapping population, and maize mutants with wild-type alleles at two known RSA genes, *DEEPER ROOTING 1* (*dro1*) and *Rootless1* (*rt1*). Comparisons of univariate and multivariate genome-wide association study (GWAS) approaches revealed that multivariate traits effectively identified phenotypic and genetic relationships that influence variations in complex root crown architecture. Phenome- and genome-wide analyses showed that 3D root models, generated through X-ray computed tomography (XRT) and digital phenotyping, captured a larger proportion of RSA trait variation compared to other root phenotyping methods, as evidenced by both genome-wide and single-gene analyses. Our results also demonstrated that the '2D multi-view' method, which increases the number of rotational viewpoints in 2D imaging, improved the capture of whole root crown information and its underlying genetic variation compared to traditional 2D single-view techniques. Among the individual root traits, root pulling force (RPF) emerged as a highly heritable estimate of RSA that identified the largest number of polymorphisms shared with 3D phenotypes. These findings suggest that complementary phenotyping approaches, particularly those leveraging higher-dimensional techniques, provide a more refined understanding of gene function and annotation of polymorphisms that influence root crown characteristics in field-grown maize.

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

## P260

## Phenotypic and photosynthetic responses of maize germplasm to waterlogging stress

(submitted by Jeonghwa Kim <jhkim@udel.edu>)
Full Author List: Kim, Jeonghwa[1]; Sosnoski, Tyler[1]; James, Randii[1]; Weldekidan, Teclemariam[1]; Mu, Qi[1]
[1] Department of Plant and Soil Sciences, University of Delaware, Newark, DE, 19716

Waterlogging, caused by frequent extreme precipitation, is a significant abiotic stress reducing crop productivity. Maize (*Zea mays* L.), a vital crop that provides over half of the world's direct and indirect calorie intake, is highly susceptible to waterlogging stress, leading to significant losses in grain yield and quality. Given the vast genetic diversity present in maize exotic germplasm, utilizing this diversity is crucial for the genetic improvement of maize to enhance resilience against waterlogging stress. In this study, we conducted a field trial using a randomized complete block design with two replications under waterlogging and control conditions to screen diverse maize accessions, including inbred lines, tropical synthetics, traditional varieties, and teosintes. Waterlogging stress was applied during the vegetative growth stage. Phenotypic traits such as flowering time, tassel length, plant height, stem diameter, and photosynthetic performance were measured to assess plant responses under the waterlogging and control conditions. Our preliminary results showed that waterlogging stress significantly affected the reproductive growth of maize by delaying days to silking and reducing tassel size. Additionally, plant height, stem diameter, and relative chlorophyll content were reduced significantly under the waterlogging condition. The observed phenotypic and photosynthetic responses provide valuable insights into maize adaptation mechanisms under waterlogging stress. The putative waterlogging-tolerant maize germplasm will serve as an important resource for further genetic and genomic research to identify genetic loci conferring waterlogging tolerance and for the genetic improvement of maize to enhance resilience against waterlogging stress.

Funding acknowledgement: Dr. Qi Mu's start-up funds from Department of Plant and Soil Sciences, University of Delaware

## P261

## Phenotypic plasticity of flowering time is associated with *cis*-regulatory elements variations

(submitted by Dongdong Li <ddli@iastate.edu>)
Full Author List: Li, Dongdong[1]; Xu, Gen[2]; Escamilla, Diana M.[1]; DeTemple, Joseph M.[1]; Guo, Tingting[3]; Vanous, Adam E.[4]; Flint-Garcia, Sherry[5]; Yang, Jinliang[2]; Li, Xianran[6]; Yu, Jianming[1]
[1] Department of Agronomy, Iowa State University, Ames, IA, USA 50011
[2] Department of Agronomy and Horticulture, University of Nebraska-Lincoln, Lincoln, NE, USA 68583
[3] National Key Laboratory of Crop Genetic Improvement, Huazhong Agricultural University, Wuhan, China 430070
[4] USDA-ARS, North Central Regional Plant Introduction Station, Ames, IA, USA 50014
[5] USDA-ARS, Plant Genetics Research Unit, Columbia, MO, USA 65211
[6] USDA-ARS, Wheat Health, Genetics, and Quality Research Unit, Pullman, WA, USA 99164

Phenotypic plasticity is the property of a given individual to produce different phenotypes in response to distinct environmental conditions. Flowering time is a typical representative trait for studying phenotypic plasticity, as it exhibits a diverse outcome of the gene-environment interplay. Gene expressions are affected by multiple genetic and environmental factors and ultimately influence phenotypes. *Cis*-regulatory elements, such as promoters, enhancers, and silencers, are non-coding sequences that impact gene expression dynamics. Linking *cis*-regulatory elements that control gene expression to phenotypic plasticity of flowering time is a challenging and promising topic in evolution and genetics. By quantifying the phenotypic plasticity of flowering time through the regression of phenotypic values against the environmental index in eight B73-teosinte introgression populations, which were evaluated for flowering time across 10 natural field conditions, two reaction norm parameters, specifically intercept (genotypic mean) and slope (plasticity), were estimated. These parameters were subsequently utilized for multi-allele QTL mapping. Genetic loci harboring domestication genes, such as *ZCN8*, *ZmCCT9*, and *ZmCCT10*, were identified for reaction-norm parameters. Only the *ZmCCT10* gene was associated with both parameters. Different reaction norms of B73 and teosinte alleles at the *ZmCCT10* locus were observed along the environmental gradient. These findings indicate that *ZmCCT10* is a gene for phenotypic plasticity and may harbor *cis*-regulatory elements sensitive to environmental stimulus. By leveraging genomic and epigenomic data, the causal variant, a ~5 kb transposable element insertion upstream of the gene body, was identified. Transposable element insertion may disrupt the potential *cis*-regulatory element through an unknown mechanism or trigger DNA methylation. This results in differential methylation levels at adjacent regions and alters the gene expression of *ZmCCT10*, causing a timing change in the floral transition. This research will provide a deeper understanding of the link between phenotypic plasticity and *cis*-regulatory variation and will facilitate the breeding of climate-resilient crops.

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

## P262

## Phenotypic selection of maize adapted to temperature extremes as a strategy to maintain productivity under global climate change, part 2

(submitted by Denise E. Costich <dc58@cornell.edu>)
Full Author List: Costich, Denise E.[1]; Welbaum, Gregory E.[2]; Dawson, Henry D.[3]; Brindisi, Lara J.[1]; Romay, M. Cinta[1]; Buckler, Edward S.[1 3 4]
[1] Institute for Genomic Diversity, Cornell University, Ithaca, NY USA 14853
[2] School of Plant and Environmental Science, Virginia Tech, Blacksburg, VA USA 24061
[3] School of Integrative Plant Sciences, Section of Plant Breeding and Genetics, Cornell University, Ithaca, NY USA 14853
[4] USDA-ARS; Ithaca, NY, USA 14853

One of the goals of the multidisciplinary, multi-institutional project called CERCA (***C**ircular **E**conomy that **R**eimagines **C**orn **A**griculture)* is to create maize cultivars that can be planted at least one month earlier than the current practice, adapted to the cool and highly variable spring temperatures that are predicted by climate change modelers to prevail in the US Midwestern Cornbelt of the future. A wide variety of strategies could promote earlier planting and field emergence. As part of the trait discovery team focusing on seed germination and early seedling growth and development, we continue to evaluate diverse maize germplasm under a range of cold soil conditions. We are using the soil-based LabField™ simulation table, an instrument designed to study seed germination behavior by creating a temperature gradient across the table, enabling controlled experiments at multiple temperatures in one trial. We present here the results of our recent trials, in which we are experimenting with a range of parameters, including soil temperature range and soil type, monitoring seed and seedling trait responses in diverse germplasm. We planted seed from CIMMYT Highland Pools plus our control (LH244) in a gradient ranging from 10.5 to 2.5ºC. Under these conditions, we observed higher emergence rates in highland lines compared to LH244. However, no seedlings emerged from soil with temperatures under 9ºC. After 38 days of cold gradient, temperature was increased to 20ºC. Most of the highland seed emerged within a week, but LH244 seed subjected to

Funding acknowledgement: United States Department of Agriculture (USDA)

## P263

## Population-level study on nitrogen stress responses in sorghum

(submitted by Harshita Mangal <hmangal2@huskers.unl.edu>)
Full Author List: Mangal, Harshita[1]; Jin, Hongyu[1]; Ji, Zhongjie[1]; Xu, Gen[1]; Rodriguez, Vladimir Torres-[1]; Swaminathan, Kankshita[2]; Yang, Jinliang[1]; Schnable, James[1]
[1] Center for Plant Science Innovation and Department of Agronomy and Horticulture, University of Nebraska−Lincoln
[2] HudsonAlpha Institute for Biotechnology, Huntsville, AL

Nitrogen plays a crucial in the growth and development of plants. However, access to nitrogen is often a limiting constraint on plant growth in natural systems and represents both a major cost, and a major source of greenhouse gas emissions, for agricultural systems. Substantial variation in productivity under nitrogen limited conditions exists in crop species. Prior studies have examined nitrogen response in sorghum, but typically with only one or several genotypes not at the population level. Understanding shared and variable responses to nitrogen deficit stress at the population level can help us to understand how plants regulate the nitrogen dependent process at the genetic level and how to improve nitrogen use efficiency in crops. Here we used RNA-seq conducted on samples collected in full and nitrogen limited conditions to study genes that respond to nitrogen deficit across a population of 360 genotypes from sorghum association panel to understand the genetic diversity of transcriptomic and phenotypic responses to nitrogen availability. We identify genes that are significant to two different treatments using differential expression analysis pathways responsive to nitrogen availability via GO expression analysis. Future steps will involve the use of eQTL analysis to identify cis-and trans regulatory elements which act either across both high nitrogen and low-nitrogen or in a treatment specific manner, as well as linking variation in transcriptional responses to nitrogen to variation in phenotypic performance (height, flowering time, chlorophyll content, grain yield, grain protein content) scored for this same population under high and low nitrogen in the field across four years of historical field trials.

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

## P264

## Pre-breeding maize for enhanced performance in no-till cover cropping systems using a teosinte synthetic population

(submitted by Collin Luebbert <cluebbert@danforthcenter.org>)
Full Author List: Luebbert, Collin[1]; Bagnall, George C.[1]; Topp, Christopher[1]; Baxter, Ivan[1]
[1] Donald Danforth Plant Science Center, St. Louis, MO, 63132, USA

Climate-smart agricultural practices are essential to mitigating climate change and addressing food security. No-till cover cropping (NT-CC) management systems benefit the environment but have been shown to reduce crop yields. Cash crops such as maize (Zea mays) have been selectively bred for high-input conditions, potentially losing genetic traits crucial for thriving in NT-CC systems. We hypothesize that genetic variation important to vigor in a NT-CC system has been lost. A synthetic maize population was designed to produce lines that have around 12% teosinte alleles, with alleles also from the Nested Association Mapping (NAM) population founders and B73. 417 double haploid lines from this population, crossed to a common parent (PHZ51), were grown in two fields in single-row plots. One field was managed with NT-CC and the other with conventional tillage practices. 3 replicate blocks were planted within each field. Stand counts were recorded tri-weekly for the first month of growing. 1 month after sowing, height and leaf-count were also recorded for all plots. A genome-wide association was then conducted for each of these phenotypes within each field as well as the difference between the control and experimental fields. Significant associations for the difference between experimental and control for height and leaf count were observed. UAV imaging using RGB, LIDAR and Hyperspectral sensors was also performed the same day as manual height and leaf count measurements. Remote sensing of stand count and height showed reasonable correlations with ground truth measurements. Future work will leverage this promising proof of concept in remote sensing of these phenotypes to increase replication. Overall, this work seeks to advance pre-breeding of maize in NT-CC systems to benefit these more environmentally sustainable agronomic systems.

Funding acknowledgement: Subterranean Influences on Nitrogen and Carbon (SINC) Center

## P265

## Predicting end-of-season Sorghum biomass from seedling-stage traits

(submitted by Vinay Chaudhari <vchaudhari@danforthcenter.org>)
Full Author List: Chaudhari, Vinay[1]; Bertollini, Edoardo[1]; Ozersky, Philip[1]; Braud, Max[1]; Eveland, Andrea L[1]
[1] Donald Danforth Plant Science Center; 975 N Warson Rd; St. Louis, MO, USA 63132

Sorghum is a promising alternative bioenergy feedstock due to its resilience to drought, heat, and low-nutrient conditions, which should enable its cultivation on marginal lands. Its extensive global germplasm, encompassing genotypes adapted to diverse geographies and climates, provides an untapped reservoir of allelic variation.In this study, we evaluated the potential of machine learning methods to predict end-of-season sorghum biomass using an extensive set of seedling-stage size and shape measurements. We collected high-throughput, above-ground phenotypic data from 285 re-sequenced sorghum lines grown at 80% soil moisture content over 11 days in the Danforth Center Bellwether controlled-environment phenotyping platform. From this temporal dataset of more than 1,000 individuals (four replicates), we extracted architectural traits using PlantCV, creating a detailed, multi-dimensional profile of plant growth and enabling the accurate detection of phenotypic variation across the sorghum panel.Several machine learning models - random forest, gradient boosting, decision tree, and feedforward neural networks - were then applied to relate seedling traits to final biomass. These models achieved robust coefficients of determination ($R^2$) with relatively low mean squared errors and root mean squared errors, demonstrating the feasibility of early biomass prediction. In addition, pan-genomic and phenotypes from controlled environment data were integrated into genomic prediction models for biomass accumulation, revealing molecular signatures associated with this trait.Overall, our work contributes to developing early selection approaches that allow breeders to discard poor-performing lines at the seedling stage, thereby reducing resource use and expediting the development of climate-resilient, high-yielding sorghum varieties for sustainable bioenergy production.

Funding acknowledgement: DOE-BER award #DE-SC0023305, USDA-NIFA funded AI Institute AIFARMS #2020-67021-32799

**P266** @LibiaGomezT

## Rust nonhost resistance responses in sorghum and maize

(submitted by Libia F. Gomez-Trejo <lgomez5@unl.edu>)
Full Author List: Gómez-Trejo, Libia F.[1 2]; Snodgrass, Claire[3]; Sigmon, Brandi[1 3]; Schnable, James C.[2 4]; Kim, Saet-Byul[1 2]
[1] Department of Plant Pathology, University of Nebraska-Lincoln, Lincoln, NE 68588, USA
[2] Center for Plant Science Innovation, University of Nebraska-Lincoln, NE 68588, USA
[3] Microbiology Program, University of Nebraska-Lincoln; Lincoln, NE 68588, USA
[4] Department of Agronomy and Horticulture, University of Nebraska-Lincoln, Lincoln, NE 68588, USA

Sorghum and maize are essential cereal crops susceptible to various fungal pathogens, including rust fungi. Common rust, caused by *Puccinia sorghi*, affects maize but does not infect sorghum, whereas sorghum rust, caused by *P. purpurea*, specifically infects sorghum, not maize. This relationship exemplifies nonhost resistance (NHR), a robust form of plant immunity that protects an entire plant species from all pathogens that affect other plant species. To investigate the mechanisms of NHR in these crops, we are screening diverse sorghum and maize lines, revealing distinct plant defense responses. In the sorghum association panel (SAP) inoculated with *P. sorghi*, disease resistance has been characterized by anthocyanin accumulation with variations ranging from numerous freckle-like lesions to no visible lesions. Sorghum produces pigmented compounds in response to fungal pathogens and previous models have implicated pathogen-associated molecular pattern (PAMP) recognition and transcriptional regulation play roles in their synthesis during biotic stress responses. In maize, the Nested Association Mapping (NAM) parent lines inoculated with *P. purpurea*, a hypersensitive response is commonly observed at pathogen entry points. This study aims to elucidate the variation in rust resistance responses within and between these crop species, highlighting the need for further investigation into the underlying resistance mechanisms of rust fungi.

Funding acknowledgement: United States Department of Agriculture (USDA)

## P267 @genomeofforrest

## Sequencing a seed bank: Assessing the utility of environmental data from CIMMYT traditional varieties for climate-adaptive maize breeding

(submitted by Forrest Li <frrli@ucdavis.edu>)
Full Author List: Li, Forrest[1 2]; Gates, Dan J[2 3]; Buckler, Edward S[4 5]; Hearne, Sarah J[6]; Ross-Ibarra, Jeffrey[2 3 7]; Runcie, Daniel E[1]
[1] Department of Plant Sciences; University of California, Davis, CA, USA 95616
[2] Department of Evolution and Ecology; University of California, Davis, CA, USA 95616
[3] Center of Population Biology; University of California, Davis, CA, USA 95616
[4] Institute for Genomic Diversity; Cornell University, Ithaca, NY, USA 14853
[5] USDA-ARS; Robert W. Holley Center for Agriculture and Health, Ithaca, NY, USA 14853
[6] International Maize and Wheat Improvement Center (CIMMYT); El Batan, Texcoco, Mexico 56237
[7] Genome Center; University of California, Davis, CA, USA 95616

Seed banks housing ex-situ collections of traditional crop varieties harbor considerable genetic variation that may be harnessed for adaptive breeding in novel climates. However, identifying adaptive loci and testing their agronomic performance in large populations in field trials is costly. In collaboration with the International Maize and Wheat Improvement Center (CIMMYT), we evaluate the comparative utility of climate and genomic data for identifying promising traditional varieties and adaptive variants to incorporate into maize breeding programs. To do so, we evaluate phenotypic data from more than 4,000 geo-referenced traditional maize varieties grown in 13 field trial environments. First, we use genotyping-by-sequencing data to predict environmental characteristics of germplasm collections to identify varieties that may be locally adapted to target environments. We also use environmental GWAS (envGWAS) to identify genetic loci associated with historical divergence along climatic gradients, such as the putative heat shock protein *hsftf9* and the large-scale adaptive inversion *Inv4m*. We then compare the value of environmental data and these envGWAS-prioritized loci to genomic data for prioritizing traditional varieties. Using prediction models such as ridge regression and random forests, we find that maize yield traits are best predicted by genomic data, and that envGWAS-identified variants provide little direct predictive information over broader patterns of population structure. Likewise, adding environment-of-origin variables does not improve yield component prediction over kinship or population structure alone, but could be a useful selection proxy in the absence of sequencing data. Finally, we extend these efforts to gene-environment association of a much larger pooled-sequencing data set of more than 15,000 traditional varieties in the CIMMYT seed bank.
Funding acknowledgement: National Science Foundation (NSF), United States Department of Agriculture (USDA), CIMMYT

## P268

## Speed breeding fast-flowering mini-maize

(submitted by Jacob Kelly <jakhdd@missouri.edu>)
Full Author List: Kelly, Jacob A[1]; Liu, Hua[2]; Yang, Bing[2]; Birchler, James A[1]
[1] Division of Biological Sciences, University of Missouri-Columbia; Columbia, MO, USA 65211
[2] Division of Plant Science & Technology, University of Missouri-Columbia; Columbia, MO, USA 65211

The Fast-Flowering Mini-Maize (FFMM) lines were created as a rapid generation model system within maize to expedite research on this species. In the realm of plant breeding, multiple research groups have developed Speed Breeding methods to reduce generation time in rice, soybean, and canola by optimizing environmental conditions. By applying Speed Breeding techniques to FFMM plants in a growth chamber, time to flowering has been reduced by 8-10 days compared to plants grown in our standard greenhouse conditions that typically proceed seed to seed in sixty days. Our standard conditions allow about six generations of FFMM in one year. By combining Speed Breeding with FFMM, it should be possible to accelerate maize research further. This project is funded by NSF PGRP 2221891.

Funding acknowledgement: National Science Foundation (NSF)

## P269

### Temporal field-based phenomics for evaluating transgenic maize under drought stress

(submitted by Juliana Yassitepe <juliana.yassitepe@embrapa.br>)
Full Author List: Pereira, Helcio[1 2]; Nonato, Juliana[1 2]; Duarte, Rafaela[1 3]; Gerhardt, Isabel[1 2 4]; Dante, Ricardo[1 2 4]; Arruda, Paulo[1 2]; Yassitepe, Juliana[1 2 4]
[1] Genomics for Climate Change Research Center, Campinas, SP, Brazil
[2] Centro de Biologia Molecular e Engenharia Genética, Unicamp, Campinas, SP, Brazil
[3] Embrapa Meio Ambiente, Jaguariuna, SP, Brazil
[4] Embrapa Agricultura Digital, Campinas, SP, Brazil

Climate change has increased the need for drought-resilient crops, yet traditional assessment methods are labor-intensive. This study utilized an unmanned aerial system (UAS) with RGB and multispectral sensors to monitor transgenic maize hybrids under irrigated and drought conditions. Machine learning models revealed strong correlations between vegetation indices and phenotypic traits, with RGB sensors outperforming multispectral sensors in trait prediction. Prediction accuracies ranged from 0.35 to 0.70 for traits like grain yield, days to anthesis, and plant height. Ridge regression and random forest models provided the best predictions. The vegetation indices NGRDI, VARI, and RCC effectively predicted and captured the plant response to drought. This study demonstrates the potential of UAS phenotyping as an efficient tool for assessing drought resilience in maize breeding programs.

Funding acknowledgement: Fapesp

## P270

### Ten years of Genomes to Fields: a collaborative corn breeding effort

(submitted by Qiuyue Chen <qchen295@wisc.edu>)
Full Author List: Chen, Qiuyue[1 2]; Holland, James B[2 3]; Kaeppler, Shawn M[1 4]; de Leon, Natalia[1]
[1] Department of Plant and Agroecosystem Sciences, University of Wisconsin-Madison, Madison, WI 53706
[2] Department of Crop and Soil Sciences, North Carolina State University, Raleigh, NC 27695
[3] USDA-ARS, Plant Science Research Unit, North Carolina State University, Raleigh, NC 27606
[4] Wisconsin Crop Innovation Center, University of Wisconsin-Madison, Middleton, WI 53562

With the rapidly growing global population and changing climate, a critical challenge for agriculture is ensuring its sustainability to meet the food supply demands. Addressing this challenge requires the development of crop varieties capable of thriving in diverse environments. The Genomes to Fields (G2F) Initiative was launched over a decade ago to tackle this issue by studying the interactions between crop genomes, environments, and management practices and their effects on crop performance. To date, G2F has evaluated approximately 190,000 field plots, involving more than 6,000 corn hybrids across over 300 unique environments in North America. The initiative has generated publicly available datasets that include genotype, phenotype, soil, weather, and metadata, offering an unparalleled resource for identifying connections between genetic variation, environmental factors, and crop performance. Here, we summarize G2F's experimental designs, germplasms, testers, environments, the questions that have been investigated over the years, and the future research opportunities it presents, reinforcing its role in advancing a sustainable agricultural system.

Funding acknowledgement: United States Department of Agriculture (USDA)

## P271

### Tensor decomposition reveals *trans*-regulated gene modules in maize drought response

(submitted by Jiawen Lu <Carmen_lujw@163.com>)
Full Author List: Lu, Jiawen[1]; Xie, Yuxin[1]; Li, Chunhui[1]; Yang, Jinliang[2]; Fu, Junjie[1]
[1] Institute of Crop Sciences, Chinese Academy of Agricultural Sciences, Beijing, China 100081
[2] Department of Agronomy and Horticulture, University of Nebraska-Lincoln, Lincoln, NE, USA 68588

When plants respond to drought stress, dynamic cellular changes occur, accompanied by alterations in gene expression, which often act through *trans*-regulation. However, the detection of *trans*-acting genetic variants and networks of genes is challenged by the large number of genes and markers. Using a tensor decomposition method, we identify *trans*-acting expression quantitative trait loci (*trans*-eQTLs) linked to gene modules, rather than individual genes, which were associated with maize drought response. Module-to-trait association analysis demonstrates that half of the modules were relevant to drought-related traits. Genome-wide association studies of the expression patterns of each module identify 286 *trans*-eQTLs linked to drought-responsive modules, the majority of which cannot be detected based on individual gene expression. Notably, the *trans*-eQTLs located in the regions selected during maize improvement tend towards relatively strong selection. We further prioritize the genes that affected the transcriptional regulation of multiple genes in *trans*, as exemplified by two transcription factor genes. Our analyses highlight that multidimensional reduction could facilitate the identification of *trans*-acting variations in gene expression in response to dynamic environments and serve as a promising technique for high-order data processing in future crop breeding.

## P272

### Testing the role of copy number variation in adaptation to drought using a pangenome approach

(submitted by Chad Soenksen <chad.soenksen@colostate.edu>)
Full Author List: Soenksen, Chad[1]; Mullen, Jack[1]; Woods, Patrick[1]; McKay, John[1]
[1] Colorado State University; Fort Collins, Colorado, United States of America 80525

Copy Number Variation (CNV) was identified as a potentially significant source of phenotypic variation long before the first genome was assembled and annotated. The extent to which variation in multiple functional gene copies exists and contributes to phenotypic variation remains underexplored, including in crops. In maize, genotype to phenotype mapping has been based on a single reference genome which fails to accurately catalog CNV. Theoretical predictions suggest that CNV can underlie phenotypic diversity via gene dosage, neofunctionalization and effects on regulatory networks. In maize, CNVs are prevalent in the dispensable and private fractions of the pangenome that may underlie population-specific adaptations. By leveraging multiple reference genomes, this research addresses the relative importance of accounting for structural genomic diversity in adaptive evolution and complex trait variation. To address this, we are utilizing available reference genomes representing diverse maize inbred lines to investigate two key questions: (1) What is the prevalence of CNV in modern maize? and (2) To what extent does CNV explain phenotypic variation in root traits? To investigate the role of CNV in complex trait variation, we are genotyping and phenotyping a double haploid mapping population derived from the inbred lines PH207 and LH127, using resequencing data aligned to diverse maize reference genomes. Our approach will enable a comprehensive identification of CNV regions, capturing structural variations often missed with a single reference genome. By associating CNV data in a mapping population with root trait measurements under well-watered and controlled drought conditions, we aim to quantify the contribution of CNV to phenotypic variation within and across environments. This research will expand our understanding of genomic diversity and phenotypic variation while aiding strategies for crop improvement.

Funding acknowledgement: National Science Foundation (NSF)

## P273

## The ZmCCT10 transcription factor promotes DNA-mediated RNA polymerase beta expression, enhancing resistance to salt stress and improving yield in maize

(submitted by Yipu Li <liyipu1987@163.com>)
Full Author List: Li, Yipu[1]; Lin, Yanan[1]; Xu, Zhenghan[1]; Tong, Lixiu[2]; Liu, Jian[1]; Gao, Julin[1]; Xu, Mingliang[2]; Yu, Xiaofang[1]
[1] Agricultural College, Region Research Center for Conservation and Utilization of Crop Germplasm Resources in Cold and Arid Areas, Inner Mongolia Agricultural University, Hohhot, Inner Mongolia, China,010018
[2] National Maize Improvement Center, China Agricultural University, Beijing, China,100193

Salinization of farmlands has become one of the major environmental stresses that adversely affects maize yield and quality. Understanding the mechanisms of salt tolerance is essential for developing salt-tolerant maize varieties, which are crucial for ensuring the sustainability of maize production and improving global grain yield. Our previous study identified ZmCCT10, a key transcription factor involved in maize growth, development, and resistance to stalk rot. In this study, we used near-isogenic lines and transgenic functional complementation plants of ZmCCT10 to confirm its role in salt tolerance. Moreover, to identify the downstream genes regulated by ZmCCT10, we conducted a DAP-Seq (DNA Affinity Purification) and RNA-Seq joint analysis. *ZmRDRPβ,* a DNA-mediated RNA polymerase, was selected as the candidate gene regulated by ZmCCT10. The interaction between ZmCCT10 and ZmRDRPβ was confirmed through yeast one-hybrid assays, luciferase reporter assays, and DNA pull-down. ZmRDRPβ is involved in ion transmembrane transport, contributing to enhance maize resistance to saline stress. The salt tolerance regulatory pathway involving ZmCCT10 and *ZmRDRPβ* provides valuable insights into the genetic mechanisms underlying maize's response to salinity and offers potential targets for the breeding salt-resilient maize hybrids.

Gene / Gene Models described: *ZmCCT10*; GRMZM2G0381691
Funding acknowledgement: National Science Foundation of China (NSFC)

## P274

## The cold case: Characterizing photosynthetic performance at suboptimal temperatures using diverse germplasm

(submitted by Emma Leary <enltz5@mail.missouri.edu>)
Full Author List: Leary, Emma N[1 3]; McCubbin, Tyler J[2 3]; Washburn, Jacob D[2 3]
[1] Division of Plant Science and Technology, University of Missouri, Columbia, MO 65211
[2] USDA-ARS Plant Genetics Research Unit, Columbia, MO 65211
[3] Interdisciplinary Plant Group, University of Missouri, Columbia, MO 65211

Nitrogen (N) availability in the soil accelerates in the spring as warming soil facilitates renewed microbial activity, whereas the demand for nitrogen by maize peaks later in the summer. This delay between peak nitrogen availability and plant uptake causes a substantial period of time during which soil N is not utilized for plant nutrition but is instead lost through N2O emissions, runoff, and leaching. Some of this N loss could be prevented by beginning the maize growing season earlier in the spring. Moving the growing season forward, however, means growing the plants at colder spring-time temperatures. This can be problematic for maize because its photosynthetic capacity declines under lower temperatures. Maize contains incredible genetic diversity and has been adapted to high elevations with low temperatures offering promise for the existence of cold tolerant photosynthetic traits. Identifying these variations could improve modern maize and, along with frost tolerance, enable earlier planting. This project seeks to discover the genetic and physiological mechanisms that influence photosynthetic performance under cold conditions. We have begun screening for germplasm that exhibits healthy photosynthesis at cold temperatures across maize genotypes, landraces, and wild relatives. Transgenic lines with changes in photosynthesis related pathways are also in development. Screening is being conducted in controlled environment chambers to simulate the cool conditions of an early spring midwest planting. Photosynthetic performance is being assessed using a Li-Cor 6800 and manual phenotyping. Preliminary results to date have identified promising germplasm for further experimentation and breeding. Ultimately, this project seeks to identify important cold tolerance traits and germplasm for introduction into modern maize breeding programs, expanding the potential growing seasons and regions for North American maize production.

Funding acknowledgement: United States Department of Agriculture (USDA), Department of Energy (DOE), Oak Ridge Institute for Science and Education

## P275

## Understanding the genetic impact of divergent selection on vegetative phase change in maize

(submitted by Heather Wodehouse <wodehouse@wisc.edu>)
Full Author List: Wodehouse, Heather C[1]; Tracy, William F[1]
[1] University of Wisconsin; Madison, Wisconsin, USA 53703

Vegetative phase change in maize is a fundamental developmental milestone during which juvenile tissue gives way to production of adult vegetative tissue. The timing of this transition impacts multiple agronomic traits such as plant maturity, architecture and response to pests. Through recurrent divergent selection of a heterozygous maize population for early and late phase change, substantial shifts in transition timing have been achieved in both directions, ranging from a last leaf with juvenile wax as early as leaf three to as late as leaf 24. While many genes influencing timing of vegetative phase change have been previously identified and characterized, mapping the specific genetic changes resulting from recurrent selection on timing of phase change could reveal novel loci. Three F2 populations derived from biparental crosses between individuals from extreme early and extreme late phase change heterozygous populations were grown and phenotyped. The last leaf with juvenile wax varied across populations, showing the variation in sampled alleles from the extreme phase change populations from which the parents were chosen. All three F2 populations are overrepresented by individuals with early phase change, suggesting that the alleles contributing to early phase change tend to exhibit dominance over alleles contributing to late phase change. These preliminary insights will be followed by F2:3 QTL mapping and population genetic analysis of the selected extreme phase change populations.

## P276

## Unlocking the secrets of ear length and frost tolerance in maize and *Tripsacum*

(submitted by YUN LUO <yl3956@cornell.edu>)
Full Author List: Yun, Luo[1 2 3]; Yu, Liu[2 3]; Jianbing, Yan[2 3]; Edward S, Buckler[1 4 5]
[1] Institute for Genomic Diversity, Cornell University, Ithaca, NY, USA 14853
[2] National Key Laboratory of Crop Genetic Improvement, Huazhong Agricultural University, Wuhan 430070, China
[3] Hubei Hongshan Laboratory, Wuhan 430070, China
[4] Section of Plant Breeding and Genetics, Cornell University, Ithaca, NY, USA 14853
[5] USDA-ARS, Robert W. Holley Center for Agriculture and Health, Ithaca, NY, USA 14853

Boosting crop yields is essential for global food security. In maize, enhancing ear length offers a promising strategy to increase grain number and yield without altering planting density. Here, we clone a quantitative trait locus (QTL), *qKB6.2a*, encoding thiamine pyrophosphokinase2 (*ZmTPK2*), which modulates grain yield by regulating florets production and ear elongation. ZmTPK2 catalyzes thiamine conversion to thiamine pyrophosphate (TPP), a vital coenzyme involved in key energy metabolism pathways. Precise manipulation of TPP levels through ectopic expression of *ZmTPK2* significantly enhances maize ear length and grain yield. Field experiments demonstrate that rare natural variants of *ZmTPK2* increase maize hybrid yields by 7.4% to 14.2%. Additionally, exogenous application of 0.04% TPP *in vitro* enhances grain yields in elite hybrids of major crops including maize, rice, and rapeseed, with yield improvements ranging from 4.5% to 9.8%. These findings provide a simple and feasible new approach for crop yield enhancement in the future.Additionally, enhancing nitrogen use efficiency is crucial for sustainable agriculture. The CERCA project initiative addresses this challenge by proposing earlier maize planting to synchronize nitrogen uptake with soil availability. *Tripsacum dactyloides*, a wild relative of maize with exceptional cold tolerance, offers a valuable genetic resource for elucidating the molecular basis of this traits. Leveraging scRNA-seq, we aim to uncover the genetic and molecular mechanism of frost tolerance in a *Tripsacum dactyloides* x *Tripsacum floridanum* F2 population. Key frost-tolerance genes identified will be integrated into maize through backcross breeding or transgenic approaches, enhancing frost resilience and nitrogen use efficiency in cultivated maize, ultimately improving crop performance and sustainability.**Key words**: Thiamine pyrophosphate (TPP), fine-regulation, grain yield, *Tripsacum*, frost tolerance

Gene / Gene Models described: *ZmTPK2*; Zm00001d037916
Funding acknowledgement: National Science Foundation (NSF)

## P277

## Unraveling a MYB transcription factor controlling plant height and involved in phenotypic plasticity in sorghum and maize

(submitted by Qi Mu <qimu@udel.edu>)
Full Author List: Mu, Qi[1]
[1] Department of Plant and Soil Sciences, University of Delaware; Newark, DE, USA 19716

Plant height is a key agronomic trait that directly reflects crop development, performance, and phenotypic plasticity across environments. Previously, we identified a plant height quantitative trait locus *qHT7.1* in sorghum involved in phenotypic plasticity by interacting with the diurnal temperature range during a critical early growth period. In this study, we positionally cloned this QTL as a MYB transcription factor controlling internode elongation, cell proliferation, and cell morphology. A 740 bp transposable element insertion in the intronic region caused a partial mis-splicing event, generating a novel transcript that included an additional exon and a premature stop codon, leading to short plant height in sorghum. The dominant allele exhibits higher expression than the recessive allele across development and internode position, while both alleles' expressions peaked at 46 days after planting and gradually decreased from the top to lower internodes. The orthologue of *qHT7.1* was identified to underlie the *brachytic1* (*br1*) locus in maize. A large insertion in exon 3 and a 160 bp insertion at the promoter region were identified in the *br1* mutant, while an 18 bp promoter insertion was found to be associated with reduced plant height in a natural recessive allele. CRISPR/Cas9–induced gene knockout of *br1* in two maize inbred lines showed significant plant height reduction. These findings revealed functional connections across natural, mutant, and edited alleles of this MYB transcription factor in sorghum and maize, offering new insights into plant height regulation and novel strategies for optimizing plant stature in crop improvements. Future investigations will focus on deciphering the mechanisms of this gene in phenotypic plasticity and its specific interaction with the diurnal temperature range.

Gene / Gene Models described: *br1 (maize); qHT7.1 (sorghum)*; Zm00001d032194; Sobic.007G137101
Funding acknowledgement: United States Department of Agriculture (USDA), Iowa State University, University of Delaware

## P278 @freeaSarahgene

## Unraveling the genetic architecture of free asparagine in maize kernels

(submitted by Sarah Fitzsimmons (Oliver) <sloxmd@umsystem.edu>)
Full Author List: Fitzsimmons (Oliver), Sarah[1]; Duong, Ha N.[1]; Shrestha, Vivek[2]; Li, Xiaowei[3]; Hirsch, Candice N.[4]; Gore, Michael A.[3]; Angelovici, Ruthie[1]; Flint-Garcia, Sherry[1 5]
[1] Division of Biological Sciences, University of Missouri, Columbia, Missouri, USA 65211
[2] Bayer Crop Sciences, St. Louis, Missouri, USA 63167
[3] School of Integrative Plant Science, Cornell University, Ithaca, New York , USA 14850
[4] Department of Agronomy and Plant Genetics, University of Minnesota, Saint Paul, MN, USA 55108
[5] Plant Genetics Research Unit, USDA-ARS, Columbia, Missouri, USA 65211

Asparagine (Asn) is a key nitrogen transport and storage molecule in plants, impacting germination and stress responses. Free Asn is also the precursor to the dietary carcinogen, acrylamide, which is formed in various cooking processes. To identify genetic factors influencing free Asn accumulation in maize kernels, we implemented and integrated GWAS, TWAS, and WGCNA with gene expression and predicted protein-protein interaction data to identify candidate genes responsible for free Asn accumulation in dry maize kernels. Of the three quantitative approaches, we obtained 778 significant associations for the GWAS and 376 for the TWAS, while the WGCNA produced 2,581 results. Our findings highlight the involvement of genes related to central metabolism and amino acids metabolism, notably, delta-1-pyrroline-5-carboxylate synthase, a key proline metabolism gene, was identified and exhibits strong interactions with all four asparagine synthases in protein-protein interaction networks. Additionally, Amino Acid Permease 6 was also identified and may be a key Asn transporter to the kernels. Surprisingly, none of the four *asparagine synthase* genes were significantly associated with Asn levels in the dry seeds. To validate these findings, we are currently analyzing a bi-parental QTL mapping population derived from high (CML14) and low (B64) Asn lines. Ultimately, this research will identify gene targets that will enable reduction of free Asn in dry kernels, which will facilitate safer maize related food products.

Funding acknowledgement: United States Department of Agriculture (USDA)

## P279

## Untangling specificity: Investigating tissue-specific responses to bacterial pathogens in maize

(submitted by Peyton Sorensen <peytonls@illinois.edu>)
Full Author List: Sorensen, Peyton L.[1]; Rapciak, Robert[1]; Mullens, Alexander C.[1]; Jamann, Tiffany M.[1]
[1] Department of Crop Sciences; University of Illinois Urbana-Champaign; Urbana, IL, United States 61801

Bacterial pathogens significantly contribute to losses in maize yields leading to lost profits and a reduction in the global food supply. Genetic resistance is an effective method to prevent yield losses, especially as there are no practical forms of chemical control for maize bacterial diseases. The cellular architecture within maize leaves creates different environments for bacteria to inhabit. Bacterial pathogens have adapted to infect different tissues, and we can infer plants will respond differently depending on the tissue type they are colonizing. I hypothesize there are tissue-specific host resistance responses. In this research we are investigating tissue-specific resistance in the hydathodes, xylem, and mesophyll. Two economically significant bacterial pathogens are being utilized. *Xanthomonas vasicola* pv. *vasculorum* (*Xvv*) is a gram-negative and motile causal agent of bacterial leaf streak. *Clavibacter nebraskensis* is a gram-positive and non-motile causal agent of Goss's wilt and leaf blight. Both pathogens cause foliar disease and can infect via hydathodes. Despite the differences between these two pathogens, similar levels of hydathode resistance have been observed in the same maize lines. A high throughput hydathode inoculation assay is being developed to investigate hydathode-mediated resistance in maize. Differential foliar resistance phenotypes have been observed for *Xvv* depending on whether infection occurs in the xylem or mesophyll tissue. A quantitative trait locus (QTL) has been detected on chromosome 4 for mesophyll resistance. We are fine-mapping mesophyll resistance. Investigating tissue-specific responses to bacterial pathogens is a significant avenue of research as it will further our understanding of the strategies maize employs to prevent infection.

Gene / Gene Models described: ; Zm00001eb200740
Funding acknowledgement: National Science Foundation (NSF), Illinois Corn Growers Association

## P280

## Using a high-throughput screening method to compare chamber and field grown VPD transpirational breakpoints in *Zea mays*

(submitted by Robert Twohey III <twohey2@illinois.edu>)
Full Author List: Twohey III, Robert J.[1]; Sen, Sabyasachi[2]; Christenson, Clay G.[3]; Calleja, Sebastian[3]; Pastor, Bryan C.[3]; Pauli, Duke[3]; Stroock, Abraham D.[4]; Studer, Anthony J.[1]
[1] Department of Crop Sciences, University of Illinois, Urbana, IL 61801, USA
[2] Sibley School of Mechanical and Aerospace Engineering, Cornell University, Ithaca, NY 14853, USA
[3] The School of Plant Sciences, University of Arizona, Tucson, AZ 85721, USA
[4] Smith School of Chemical and Biomolecular Engineering, Cornell University, Ithaca, NY 14853, USA

Rising atmospheric temperatures and increasingly sporadic precipitation are intensifying droughts during our crop growing seasons. Both increased temperatures and drier growing conditions result in a higher atmospheric vapor pressure deficit (VPD). Atmospheric VPD is defined as the difference between the amount of moisture in the air and its saturation point. As VPD increases, the atmospheric water demand intensifies, resulting in increased plant water loss through transpiration. Past studies have identified VPD breakpoints in maize which are observed when the linear transpirational response to increasing VPD changes in slope. We previously developed a high-throughput screening protocol for measuring VPD breakpoints. This protocol revealed consistent variation in the occurrence of VPD breakpoints and the severity of VPD required to provoke a stomatal response in 28 chamber grown *Z. mays* lines. In addition, a *slac1-2* mutant that is unable to close its stomata did not show a breakpoint, identifying stomatal dynamics as the primary driver of VPD breakpoints in *Z. mays*. Here we present further characterization using a nano particle called Aquadust to measure water potential in the leaf during VPD curves. Also, to test whether growth chamber VPD breakpoints accurately represent the dynamic response of field grown plants, we evaluated eight genotypes in a Maricopa, Arizona field trial. Similar response patterns to increasing VPD were observed in the field compared to the growth chambers. Interestingly, the *slac1-2* mutant showed a significantly less severe response to VPD in the field compared to wild-type. Observing repeatable VPD breakpoint responses in a field environment supports further investigation into the genetic regulation of this trait. Improving our ability to measure VPD breakpoints and identify genetic mechanisms regulating VPD response will provide new insights into stomatal dynamics and the programing of water use efficiency in *Z. mays*.
Gene / Gene Models described: *slac1*; GRMZM2G106881
Funding acknowledgement: National Science Foundation (NSF)

## P281 @cornontherocks

### Validating a lab-scale ethanol production method for exploring metabolites in craft distilling

(submitted by Zachary Traylor <zbtyxb@umsystem.edu>)
Full Author List: Traylor, Zachary B.[1]; Somavat, Pavel[2]; Hinegardner, Gary[3]; Braun, David M.[1]; Flint-Garcia, Sherry[4]
[1] MU Division of Biological Sciences; Columbia, MO, 65201
[2] MU Department of Agricultural Engineering; Columbia, MO, 65201
[3] Woodhat Spirits; New Florence, MO, 63363
[4] USDA-ARS Plant Genetics Research Unit, Columbia, MO, 65201

Maize diversity has become a prominent research area to incorporate novel alleles not currently available in elite inbreds. One source of maize diversity we could leverage is heirlooms/landraces which are locally adapted populations grown before the development of inbreds and hybrids. With new biochemical and genomic tools, we can study new traits to improve maize using heirlooms, such as improving its nutritional quality, or making populations better suited for food products rather than for feed or fuel. Specifically, we aim to optimize maize for the whiskey industry by producing metabolically unique lines from heirlooms that offer new flavor profiles not currently available in commercial germplasm. To do this, we are conducting a QTL analysis to find genomic regions associated with specific metabolites connected to flavor. But first, we need to establish a high-throughput method for converting the heirloom grain into ethanol. Current methods in the literature outline ways to convert starch to ethanol using a multitude of grains in low quantities, but none have been described for the dry grind method using maize in the American whiskey industry. Here we developed a method to efficiently produce whiskey using less than 100 grams of maize and have validated this method using spectrophotometric and molecular quantifications of ethanol and sugar. This platform will allow us to conduct our QTL studies with maize heirlooms and map traits associated with flavor.

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

## P282

### Variation in temporal growth patterns of inbred lines and hybrid offspring

(submitted by Veronica Justen <veronica.justen@uwrf.edu>)
Full Author List: Justen, Veronica L.[1]; Sweet, Dorothy D.[2]; Cooper, Julian[2]; Hirsch, Candice N.[2]
[1] University of Wisconsin-River Falls, River Falls, Wisconsin, 54022
[2] University of Minnesota, Saint Paul, Minnesota, 55108

Plant height, canopy cover and related growth patterns are important agronomic traits that can serve as indicators of plant health and yield making them useful selection criteria in maize breeding. High temporal resolution data can be used to resolve plant height and canopy cover growth patterns. To assess the variation in growth patterns between inbreds and hybrids, plant height and canopy cover were evaluated from 510 inbred lines and 840 derived hybrids using weekly images captured from unoccupied aerial vehicles. Images were captured from emergence until approximately three weeks (500 GDU) post-anthesis (14 flights in 2022, 19 in 2023, and 13 in 2024). A comparison of plant height and canopy growth curves between parent inbred lines and hybrid combinations, differences in partitioning of phenomic variation between inbreds and hybrids, and the genetic architecture underlying heterosis of growth curves will be presented.

## P283

## *Zea diploperennis* perennial regrowth QTL *regrowth1* and *regrowth3* control life history traits

(submitted by Kyle Swentowsky <swentow@cshl.edu>)
Full Author List: Swentowsky, Kyle W[1]; Jackson, David[1]
[1] Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 11724

Perennial plants regrow for multiple years and perennial crops are sustainable agriculture systems. The teosinte species *Zea diploperennis* is perennial and forms fertile hybrids when crossed with maize. Using *Z. diploperennis*/maize mapping populations, perennial regrowth was mapped to three dominant loci called *Regrowth1* (*Reg1*), *Reg2*, and *Reg3* on chromosomes 2, 7, and 8, respectively. Perenniality is a complex trait thought to involve the convergence of many loci that independently affect phenology, physiology, and meristem traits. We measured several traits in introgressed populations that were segregating *Reg1* and *Reg3* and determined that *Z. diploperennis* alleles of *Reg1* and *Reg3* significantly delay flowering time, *Reg3* instills stay-green, and neither locus influences tiller number. We collected axillary meristems (AMs) and leaf tips of WT, *Reg1*, *Reg3*, and *Reg1 Reg3* individuals at around the V10 stage for RNA-seq analysis. Consistent with our measurements of stay-green, we observed senescence-associated marker genes were downregulated in Reg3 but not Reg1 leaves. We also used this dataset to look for differentially-expressed genes in the QTL intervals. Our preliminary analysis has led to several candidate genes that are involved in flowering, sugar transport, and hormone signaling. Overall, these data support the idea that perenniality is controlled in part by altering phenology traits and may lead to interesting candidate genes or gene modules that will aid in perennial maize breeding. We are also testing a classical hypothesis that *Indeterminate1* (*Id1*) and *Grassy tillers1* (*Gt1*) contribute to perenniality. Using knowledge of *cis*-regulatory element locations provided by Conservatory, we have generated *id1* promoter-edited alleles that have weaker flowering-time phenotypes compared to the *id1* null allele. We will combine weak alleles of *id1*, *gt1* with *Reg1* and *Reg3* alleles from *Z. diploperennis* to test if this combination is sufficient to reproduce perennial regrowth.

Funding acknowledgement: National Science Foundation (NSF)

# P284

## ZmIRA1: A novel genetic factor of root system architecture in maize identified by GWAS in a long-term selection population for kernel nitrogen content

(submitted by Michelle Cho <mcho@danforthcenter.org>)
Full Author List: Cho, Michelle[1 2]; Liu, Zhengbin[3]; Gunn, Shayla[2]; Lebow, Clara[2]; Duncan, Keith[2]; Yang, Bing[2 4]; Moose, Stephen[5]; Topp, Christopher[2]
[1] Division of Biology and Biomedical Studies, Department of Biology, Washington University in St. Louis, St. Louis, MO, 63110 USA
[2] Donald Danforth Plant Science Center, St. Louis, MO, 63132 USA
[3] Partek Inc, Chesterfield, MO, 63005 USA
[4] Division of Plant Sciences, Bond Life Sciences Center, University of Missouri, Columbia, MO 65211, USA.
[5] Department of Crop Sciences, University of Illinois Urbana-Champaign, Urbana, IL 61801, USA.

Root systems are critical for plant health and survival but have been understudied compared to their above-ground counterparts. Recent advancements in technology for capturing three-dimensional root system architecture (RSA) and the identification of genetic factors influencing RSA have become areas of increasing research focus. This study utilized the Illinois Long Term Protein Selection (ILTPS) population, which has undergone over a century of selection for high and low kernel protein content. The prolonged selection for kernel protein content may have indirectly influenced root phenotypes associated with nitrogen uptake. Using the Illinois Protein Strain Recombinant Inbreds (IPSRIs), a recombinant inbred population derived from the ILTPS, we conducted a multi-year field campaign to assess root traits and perform a genome-wide association study (GWAS). This analysis identified a gene, designated as Ideal Root Architecture 1 (ZmIRA1), which is known to function in central carbon-nitrogen metabolism. Nanopore sequencing revealed multiple indels in the promoter region of ZmIRA1, while the coding region remained conserved between the Illinois High Protein (IHP1) and Illinois Low Protein (ILP1) inbred lines. Subsequent analysis confirmed differential expression levels between these lines. Field experiments conducted in 2020 and 2022 demonstrated significant phenotypic differences between plants possessing the IHP1 or ILP1 allele. To further elucidate the function of ZmIRA1, we generated a CRISPR-Cas9 knockout mutant in a B73 background. These mutants were grown to full maturity under field conditions in 2024. Phenotypic analysis revealed that the mutants exhibited reduced overall size and accelerated development compared to wild-type controls. The identification of ZmIRA1 and its potential role in root development opens new avenues for improving maize nitrogen use efficiency and yield.

Funding acknowledgement: National Science Foundation (NSF)

# P285
## AGO2 and AGO3 regulate RNAi fidelity by suppressing RNA-directed DNA methylation

(submitted by Jason Lynn <jlynn@cshl.edu>)
Full Author List: Lynn, Jason[1]; Ernst, Evan[1]; Cahn, Jonathan[1]; Martienssen, Robert[1]
[1] HHMI/Cold Spring Harbor Laboratory; Cold Spring Harbor; New York; USA 11724

RNAi is a conserved mechanism for gene silencing consisting of two pathways that differ profoundly in their persistence– post-transcriptional gene silencing (PTGS) by small RNA-directed cleavage/translational inhibition of mRNA, or transcriptional gene silencing through DNA and histone methylation (TGS/RdDM), forming meiotically heritable heterochromatin. The silencing fate of a small RNA relies on its interaction with a family of small RNA-guided RNA-endonucleases, ARGONAUTEs (AGO), making small RNA sorting a key decision point in preventing heritable silencing and ensuring RNAi fidelity. The partitioning of RNAi occurs largely through nuclear localization of TGS/RdDM clade AGOs (AGO4/6/9) and cytosolic localization for PTGS clade AGOs (AGO1/2/3/5/7/10), however all AGOs are loaded with small interfering RNA in the cytoplasm before localizing to their target. How the cell attains RNAi fidelity in the context of dynamic environment and how this affects epigenetic inheritance remain important open questions in molecular biology. Using Arabidopsis I found that duplicated linked paralogs AGO2 and AGO3 are required to prevent ectopic TGS via the 24-nt siRNA AGO4/6/9 RdDM pathway during heat stress, causing aberrant gene expression and leading to defects in the recovery of *ago2ago3* mutant plants subjected to a thermotolerance assay. In addition, I found that *ago2ago3*-dependent thermosensitivity is affected by developmental stage, small RNA abundance and composition, and competing AGO dosage as indicated from time course and genetic experiments. In maize, CRISPR Cas9 targeting of AGO2/3 homologs ZmAGO2A/ZmAGO2B triggers spontaneous silencing and paramutation of the epigenetically unstable *b1* locus while targeting other maize AGOs does not, supporting a conserved function for AGO2/3 in suppressing RdDM. Together, these results reveal a novel role for AGO2 and AGO3 in heat stress and suggest that AGO2/3 clade proteins are master regulators of RNAi through substrate competition with canonical TGS and PTGS AGO effectors.

Funding acknowledgement: National Science Foundation (NSF), HHMI

## P286
## Active LTR transposable element annotation via a computational pipeline - LTRAnnotator

(submitted by Patrick Gardner <gardnerp1@montclair.edu>)
Full Author List: Gardner, Patrick L[1]; Isles, Taylor[2]; Singh, Angadh[3]; Du, Chunguang[4]
[1] Montclair State University, Montclair, NJ, United States 07043
[2] University of Missouri, Columbia, MO, United States 65211
[3] Wayne Valley High School, Wayne, NJ, United States 07470
[4] Department of Biology and Molecular Biology, Montclair State University, Montclair, NJ, United States 07043

Long terminal repeat retrotransposons (LTRs) are a class of transposable elements that copy and reintegrate themselves throughout a genome, proliferating over time. This process has resulted in LTRs composing a significant proportion of many eukaryotic genomes, from roughly 8% in humans, to 75% in maize. Each copy, however, is likely to mis-replicate, as reverse-transcriptase lacks proofreading functionality, and additionally may mutate over time within a particular locus. These factors can rapidly render a given copy of an LTR inactive, with mutations making the copy incapable of further retrotransposition. Because of this, most LTRs in a given genome are nonfunctional, with only a minute fraction of copies of an LTR actively propagating. LTR_FINDER is a publicly available tool that identifies potential LTRs from gene sequences. However, it does not effectively discriminate between intact and degraded LTRs. To address this, a supplemental tool was developed to filter the outputs of LTR_FINDER, and present only fully intact, active LTRs, termed LTR_ANNOTATOR. LTR_ANNOTATOR employs a local alignment between the ends of each candidate sequence to define tentative bounds for the repeats, then refines those bounds by identifying other critical elements of the LTR within set ranges of the initial positions. Once refined bounds are identified, all reading frames of the candidate sequence between the identified repeats are searched for amino acid motifs of each requisite enzyme, assembled from documented LTRs in diverse plant species. By setting more stringent constraints on identity matching, the degraded LTRs identified by LTR_FINDER are discarded, allowing for efficient assaying of active LTRs using a wholly computational approach. Outputs are readily assessed using the secondary rich text output file, which presents identified conserved LTR sequences with color-coded highlighting of necessary elements and protein domains, to ensure accuracy and aid in classification of LTR superfamily.

## P287 @yum_caax
## Adapting Fiber-seq for mapping chromatin accessibility in maize under cold stress

(submitted by Carmen Rodriguez <rodriguez.1523@osu.edu>)
Full Author List: Rodriguez, Carmen M[1]; Ou, Shujun[1]
[1] Department of Molecular Genetics, Ohio State University, Columbus, OH, 43210

Cold stress at the seedling stage can reduce maize yield and quality by disrupting critical physiological processes, including photosynthesis, cell membrane integrity, and nutrient uptake. As global temperatures fluctuate and extreme weather events become more frequent, understanding how crops like maize respond to environmental stress is crucial for ensuring sustainable food production. Long terminal repeat retrotransposons (LTR-RTs) make up a large portion of the maize genome and have been shown to influence gene expressions and epigenetic modifications through chromatin remodeling. However, their chromatin accessibility dynamics in regulating plant responses to cold stress are not yet fully understood. A major challenge in accessing the chromatin status of LTR-RTs is their repetitive nature, preventing accurate mapping and analyzing using short-read sequencing methods. To address this, we are adapting the new Fiber-seq technology for mapping chromatin accessibility. Through the introduction of m6A methylations to open chromatin following long-read sequencing, Fiber-seq can detect the introduced m6A to reveal chromatin accessibility and detect native 5mC epigenetic modifications. Thus, Fiber-seq allows accurate assessments of chromatin openness among repetitive genomic regions. To date, we have successfully isolated high-quality nuclei from maize seedlings and identified an LTR family for focused investigation. Ongoing efforts include scaling up nuclei isolation to meet experimental requirements and validating Fiber-seq labeling of chromatin accessibility in these nuclei. This research provides the groundwork for understanding chromatin remodeling of LTR-RTs in maize cold stress responses. Future directions will explore the functional impact of LTR-RTs for their regulatory potential on cold-responsive genes.

Funding acknowledgement: National Science Foundation (NSF)

## P288
## An epigenetic basis for inbreeding depression in maize

(submitted by Jeff Chen <zjchen@austin.utexas.edu>)
Full Author List: Chen, Z. Jeffrey[1]
[1] The University of Texas at Austin

Inbreeding depression is a widespread across plant and animal kingdom and may arise from exposure of deleterious alleles and/or loss of overdominant alleles resulting from increased homozygosity. However, these genetic models cannot fully explain the phenomenon. For example, yield in maize inbred lines continues to decline even after 10 generations of self-pollination. Here we present an epigenetic basis of inbreeding depression in maize. Teosinte branched1/Cycloidea/Proliferating-cell-factor (TCP) transcription factors control plant growth and development. During successive inbreeding among highly inbred lines, thousands of genomic regions across TCP-binding sites (TBS) are hypermethylated through the H3K9me2-mediated pathway. These hypermethylated regions are accompanied by decreased chromatin accessibility, increased levels of the repressive histone marks H3K27me2 and H3K27me3, and reduced binding-affinity of maize TCP proteins to TBS. Consequently, hundreds of TCP-target genes involved in mitochondrion, chloroplast, and ribosome functions are downregulated, leading to reduced growth vigor. Conversely, random matting can reverse the corresponding methylation sites and TCP-target gene expression, restoring growth vigor. These results support a unique role of reversible epigenetic modifications in inbreeding depression in maize.

## P289 @KhanguraRajdeep
## An exapted transposase modifies the *Mu*-suppressible allele of a lesion-forming UROPORPHYRINOGEN III SYNTHASE mutant

(submitted by Rajdeep Khangura <rkhangur@purdue.edu>)
Full Author List: Khangura, Rajdeep S[1 2]; Zhang, Xinyan[3 4]; Kaur, Amanpreet[1 2]; Lisch, Damon[2 3]; Dilkes, Brian P[1 2]
[1] Department of Biochemistry, Purdue University; West Lafayette, Indiana, USA 47907
[2] Center for Plant Biology, Purdue University; West Lafayette, Indiana, USA 47907
[3] Department of Botany and Plant Pathology, Purdue University; West Lafayette, Indiana, USA 47907
[4] Agricultural Genomics Institute at Shenzhen, Chinese Academy of Agricultural Sciences; Shenzhen, Guangdong, China 518120

Porphyrins include energy-transferring molecules present in all life and required for metabolism and adaptive responses to the environment. These include siroheme, cobalamin, heme, bilins, and chlorophylls. Three enzymes in porphyrin biosynthesis are present in all living things: porphobilinogen synthase, hydroxymethylbilane synthase, and uroporphyrinogen III synthase (UROS). Here, we report two semi-dominant mutant alleles at the only UROS homolog in maize. Both semi-dominant alleles carry *Mutator* transposable element insertions in non-coding sequences and result in light-dependent lesion formation in leaves. The gene is essential, and crosses between the two alleles result in a yellow-seedling lethal phenotype, similar to the recessive phenotypes of loss-of-function alleles in genes encoding the prior and subsequent enzymatic steps in the porphyrin pathway. The expression of lesions in these *Mu* insertion alleles is modulated by the epigenetic state of their *Mutator* transposons, a phenomenon called *Mu*-suppressibility. Epigenetic silencing of *Mutator* transposition activity also suppresses the lesion phenotypes of both alleles. We used the stronger allele to carry out a screen for natural variants that modify the lesion phenotype in a Genome Wide Association population made of F1 families from crossing the semi-dominant mutant to hundreds of maize inbred lines. This could detect loci affecting variation in porphyrin biosynthesis as well as mutator activity. Alleles linked to genes in the porphyrin pathway had small effect on lesion severity but included modification in cis by alleles at the *uros* gene itself. By contrast, the most signification association was encoded by an exapted MuDR family transposase which regulated lesion severity and is polymorphic in maize. This novel transposon-derived modifier exapted at least 650 kya, prior to the split of *Tripsacum* and *Zea*. This work demonstrates a new aspect of *Mutator* through the study of *Mu*-suppressible alleles and the discovery of a transposon-derived trans-regulator of mutator encoded by an exapted transposase.

Gene / Gene Models described: *uros*; Zm00001d027950
Funding acknowledgement: National Science Foundation (NSF), United States Department of Agriculture (USDA), Department of Energy (DOE)

## P290

## *BonnMu* – A resource for functional genomics in maize (*Zea mays* L.)

(submitted by Xuelian Du <xuelian@uni-bonn.de>)
Full Author List: Du, Xuelian[1 2]; Win, Yan Naing[1 2]; Stöcker, Tyll[3]; Schoof, Heiko[3]; Hochholdinger, Frank[1]; Marcon, Caroline[1 2]
[1] Institute of Crop Science and Resource Conservation, Crop Functional Genomics, University of Bonn, Bonn 53113, Germany
[2] Institute of Crop Science and Resource Conservation, BonnMu: Reverse Genetic Resources, University of Bonn, Bonn 53113, Germany
[3] Institute of Crop Science and Resource Conservation, Crop Bioinformatics, University of Bonn, Bonn 53115, Germany

The *BonnMu* resource is a public transposon-tagged population designed for reverse and forward genetics studies in maize (*Zea mays* L.). The resource was developed at the University of Bonn (Germany) by crossing an active *Mutator* (*Mu*) stock with dent (B73, Co125) and flint (DK105, EP1, and F7) germplasm, resulting in the generation of 8064 mutagenized *BonnMu* $F_2$-stocks[1,2,3]. The *Mu*-tagged *BonnMu* $F_2$-stocks have insertions in 83% of all annotated maize gene models. *Mu* insertion positions and photos of the seedling phenotypes of the segregating *BonnMu* $F_2$-stocks are deposited in the Maize Genetics and Genomics Database (MaizeGDB), and seeds are available to the research community. Downstream examination of the presumptive germinal *BonnMu* insertions shows that 94% occur in genic regions, while only a small fraction of 6% are found in non-coding intergenic sequences of the genome. Consistently, *Mu* insertions predominantly align with gene-dense chromosomal arms. In total, 42% of all *BonnMu* insertions are located in the 5' untranslated region of genes, corresponding to accessible chromatin. In summary, our European *BonnMu* resource provides broad coverage of maize genes across two major germplasm groups, making it a valuable tool for functional genomics research. Instructions for ordering *BonnMu* $F_2$-stocks are available on our website at: https://www.bonnmu.uni-bonn.de.References:[1] Marcon C, et al. (2020). *BonnMu*: A sequence-indexed resource of transposon-induced maize mutations for functional genomics studies. Plant Physiol., 184, 620-631.[2] Win YN, et al. (2024). Expanding the *BonnMu* sequence-indexed repository of transposon induced maize (*Zea mays* L.) mutations in dent and flint germplasm. Plant J., 120, 2253-2268.[3] Marcon C, et al. (2024). *BonnMu*: a resource for functional genomics in maize (*Zea mays* L.). Cold Spring Harb Protoc; doi:10.1101/pdb.top108465.

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## P291 @OsuOuLab

## Cis-regulatory elements in maize early development derived from long terminal-repeat retrotransposons.

(submitted by Caleb Gooden <gooden.67@osu.edu>)
Full Author List: Gooden, Caleb[1]; Ou, Shujun[1]
[1] Department of Molecular Genetics, The Ohio State University, Columbus, OH, USA, 43210

Gene transcriptional regulation is one of the fundamental approaches for young plants to cope with worsening global environmental conditions and maintain active development. Long terminal-repeat retrotransposons (LTR-RTs) are mobile genetic components that constitute 75% of the maize genome. They are prevalent in gene promoter and coding regions and may provide additional regulatory elements for the orchestration of gene expression. It is known that LTR-RTs can become transcriptionally activated under certain conditions, reportedly enhancing downstream gene expression or providing alternative promoters for transcription initiation. This is made possible through their characteristic LTRs, which contain putative enhancers and promoters in the U3 subregion for RNA Polymerase II recruitment. The U3 subregion is highly uncertain and diverse among LTR-RT families, and a knowledge gap persists concerning the contribution of LTR-RTs during maize early development despite evidence in other organisms. Using a custom analysis method based in Linux and R to compile and reprocess publicly available histone modification, DNA methylation, Smar2C2 transcription start site sequencing, and long-read transcriptomic data of several maize tissue types, I identified candidate LTR-RTs with multi-omics evidence implicating active transcription and revealing promising boundaries for the U3 promoter. Analyzing RNA-seq data with this method for potentially active elements in six tissues spanning maize developmental stages, I observed three distinct types of transcripts with regulatory implication originating from LTR-RTs. Using a custom pipeline to classify transcripts, I found differentiated enrichment of the LTR-RT transcript types across different stages of maize development, particularly between reproductive and mature tissues. Future work will focus on defining U3 boundaries of transcriptionally active LTR-RTs with Nanopore long reads and identifying common regulatory features of LTR-RTs involved in early maize development.

Funding acknowledgement: National Institutes of Health (NIH)

**P292** @jon_cahn/@joncahn.bsky.social

## Enhancers and enhancer RNAs recapitulate the domestication focus on maize ears

(submitted by Jonathan Cahn <cahnjonathan@gmail.com>)
Full Author List: Cahn, Jonathan[1]; Regulski, Michael[2]; Lynn, Jason[1]; Ernst, Evan[1]; de Santis Alves, Cristiane[1]; Ramakrishnan, Srividya[3]; Chougule, Kapeel[2]; Wei, Xuehong[2]; Lu, Zhenyuan[2]; Xu, Xiaosa[2 4]; Drenkow, Jorg[2]; Kramer, Melissa[2]; Seetharam, Arun[5]; Hufford, Matthew B.[5]; McCombie, W. Richard[2]; Ware, Doreen[2 6]; Jackson, David[2]; Schatz, Michael C.[2 3]; Gingeras, Thomas R.[2]; Martienssen, Robert A.[1]
[1] Howard Hughes Medical Institute, Cold Spring Harbor Laboratory; 1 Bungtown Road, Cold Spring Harbor, NY, USA 11724
[2] Cold Spring Harbor Laboratory; 1 Bungtown Road, Cold Spring Harbor, NY, USA 11724
[3] Johns Hopkins University; 1900 E. Monument Street, Baltimore, MD, USA 21205
[4] Department of Plant Biology, University of California, Davis, CA 95616, USA
[5] Department of Ecology, Evolution, and Organismal Biology, Iowa State University, Ames, IA, 50011
[6] USDA ARS Robert W. Holley Center for Agriculture and Health Cornell University, Ithaca, New York, USA

Native American farmers domesticated maize from teosinte by focusing on traits such as increased kernel row number, loss of the hard fruit case, lack of dissociation from the cob upon maturity, and fewer tillers. Molecular approaches have identified key transcription factors (TFs) involved in the development of these traits, yet the complex regulatory network at play is still being unraveled. Using transcriptomic and epigenetic datasets generated by the MaizeCODE initiative, we identified active enhancers in four tissues of four different inbreds, including TIL11, an inbred line from *teosinte parviglumis*. Importantly, thousands of these enhancers were expressing bidirectional enhancer RNAs (eRNAs), molecules that are capped and polyadenylated. We showed that production of these bidirectional eRNAs is associated with a larger number of TF binding sites and with promoting higher transcriptional activity. However, they are also associated with higher DNA methylation and 24-nt small RNA levels at their boundaries. While the role of these eRNAs remains to be deciphered, we hypothesize that they are implicated in RNA-directed DNA methylation acting at the boundaries of these enhancers to silence the neighboring TEs. Additionally, we revealed that these enhancers have been actively selected during Zea evolution, especially those promoting expression in immature ears – corroborating the less conserved expression profile of this tissue between teosinte and modern maize. These enhancers are prime candidates for understanding the molecular signatures of domestication and could be leveraged for further adapting maize to the current climate crisis.

Funding acknowledgement: National Science Foundation (NSF), HHMI

# P293

## Functional characterization of the *dizzy1* (*diz1*) dwarf maize mutant from the *BonnMu* resource

(submitted by Xuelian Du <xuelian@uni-bonn.de>)
Full Author List: Du, Xuelian[1 2]; Suresh, Kiran[3]; Schreiber, Lukas[3]; Hochholdinger, Frank[1]; Marcon, Caroline[1 2]
[1] Institute of Crop Science and Resource Conservation, Crop Functional Genomics, University of Bonn, Bonn 53113, Germany
[2] Institute of Crop Science and Resource Conservation, BonnMu: Reverse Genetic Resources, University of Bonn, Bonn 53113, Germany
[3] Institute of Cellular and Molecular Botany, Ecophysiology of Plants, University of Bonn, Bonn 53115, Germany

The *BonnMu* resource, established at the University of Bonn in Germany, is a sequence indexed collection of maize *Mutator* transposon-induced mutants, designed for forward and reverse genetics studies[1,2]. The novel monogenic recessive mutant *dizzy1* (*diz1*), with a twisted shoot and root phenotype, was identified in a segregating $F_2$-family from a forward genetics screen of the *BonnMu* population. Histological analyses revealed that *diz1* mutants have enlarged upper epidermal cells in the leaf and irregular cortical cells in the primary root compared to the wild type (WT). Combining Evans blue and 2,3,5-Triphenyltetrazolium chloride staining of primary roots demonstrated that *diz1* mutants exhibit decreased cell viability, as indicated by elevated cell membrane permeability and cell metabolic activity. To determine DIZ1-dependent gene expression patterns, we performed an RNA-Seq analysis on the primary roots of *diz1* mutants and WT. In total, 4,378 genes were differentially expressed in the *diz1* mutant. Consistent with the functional enrichment analysis of the differentially expressed genes, hydrogen peroxide and superoxide levels were measured using 3,3'-diaminobenzidine and nitro blue tetrazolium staining, respectively. These measurements, along with lignin quantification, revealed increased levels of reactive oxygen species and lignin content in the primary root of the *diz1* mutant. Our findings contribute to the functional analysis of the dwarf mutant *diz1* and aim to unravel the molecular networks associated with the candidate gene underlying its phenotype.References:[1] Marcon C, et al. (2020). *BonnMu*: A sequence-indexed resource of transposon-induced maize mutations for functional genomics studies. Plant Physiol., 184, 620-631.[2] Win YN, et al. (2024). Expanding the *BonnMu* sequence-indexed repository of transposon induced maize (*Zea mays* L.) mutations in dent and flint germplasm. Plant J., 120, 2253-2268.

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## P294

## Genetic perturbation of the *Mu*-accessible chromatin landscape

(submitted by Damon Lisch <damon.lisch@gmail.com>)
Full Author List: Lisch, Damon[1]; Wu, Shan[2]; McCarty, Donald R[2]
[1] Purdue University; West Lafayette, Indiana, 47907
[2] University of Florida; Gainesville, Florida 32611

The fate of transposons is closely tied to fate their hosts. Because of this, transposon insertion preferences are far from random. This is particularly true of *Mu* transposons of maize, which exhibit extraordinarily precise targeting of particular regions of the genome. *In vivo* profiling of chromatin accessibility using *Mu* transposons provides a uniquely quantitative probe of chromatin states associated with genes expressed at high levels in dividing, undifferentiated cells. Hence, *Mu*insertion frequency measured in somatic tissues of *Mu*-active plants reveals information not readily assembled from other chromatin and accessibility data. We compared leaf somatic *Mu* insertion profiles of plants in a *Mu*-active population segregating the *Kn1-0* over-expression allele of *Knotted1*, a key transcriptional regulator of meristem activity. A sliding window scan of wild type and *Kn1-0* genomes identified 3,810 regions with significantly more *Mu* insertions in *Kn1-0* plants. The 1,559 genes with enhanced insertions in the mutants are enriched for KN1 targets, other developmental regulators, and SNPs associated with leaf phenotypes, consistent with retargeting of *Mu* to developmentally relevant genes in mutant tissue. Affected genes include *Kn1* itself, as well as group of syntenic *Blade on Petiole* (*BOP2*) transcription factor genes, where we find a striking association with intragenic conserved non-coding sequences (CNSs). More than half of retargeted regions in *Kn1-O* tissues overlap accessible chromatin regions in the single-cell ATAC-seq atlas. Strikingly, these are regions that are enriched for accessible regions detected in multiple cell types including meristem cells, and enhanced targeting of constitutive accessible chromatin regions in *Kn1-0* leaves mirrors *Mu*'s preference for highly expressed genes with broad tissue specificities. We suggest that these retargeted regions belong to a core set inherited by diverse cell types from undifferentiated progenitors in which *Mu* is most active and that *Kn1-0* enhances targeting of this core set by delaying differentiation.

Gene / Gene Models described: *Kn1*; Zm00001eb055920
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## P295

### Identifying novel transposable element insertions from short-read data using SWIF-TE

(submitted by Claire Menard <menar060@umn.edu>)
Full Author List: Menard, Claire C[1]; Catlin, Nathan S[2 3]; Platts, Adrian E[2 3]; Qiu, Yinjie[4]; Roback, Emma Y[5]; Munasinghe, Manisha[1]; Springer, Nathan[6]; Josesphs, Emily B[2 3]; Hirsch, Candice N[1]
[1] Department of Agronomy and Plant Genetics, University of Minnesota, St Paul, MN, 55108, USA
[2] Department of Plant Biology, Michigan State University, East Lansing, MI, 48824, USA
[3] Ecology, Evolution, and Behavior Program, Michigan State University, East Lansing, MI, 48824, USA
[4] Minnesota Supercomputing Institute, University of Minnesota, Minneapolis, MN, 55455, USA
[5] Department of Ecology, Evolution, and Behavior, University of Minnesota, St Paul, MN, 55108, USA
[6] Department of Plant and Microbial Biology, University of Minnesota, Minneapolis, MN, 55455, USA

Transposable element (TE) insertion polymorphisms (TIPs) are TE sequences not found in the same location between individuals. TIPs have the potential to contribute to phenotypic variation and genome evolution. Historically, the major challenges with genome-wide characterization of TIPs at a population scale have been access to high quality genome assemblies, precise annotation of reference transposable elements, and a lack of algorithms that could accurately use this information with low cost sequence data to identify novel insertions. While technological advances have made it easier to develop high-quality assemblies and reference TE libraries, there are still few algorithmic tools that can use this information to call TIPs using affordable short-read sequencing data. Here, we describe a fast and memory efficient tool to identify novel TIPs from the alignment of short read sequences to a reference genome assembly and a reference library of canonical TE sequences. SWIF-TE (Short-read Whole-genome Insertion Finder for Transposable Elements) was built to balance computational resources and accuracy for practical use in species with large genomes. SWIF-TE was able to identify 1,438 insertions in Oh43 compared to the B73 reference genome assembly at a precision rate of 27% using just 0.10 Gb of memory and 0.82 hours of runtime from 15x resequencing data of a maize inbred line with a genome size of approximately 2.4 Gb. At the population level, using 15x resequencing data from 25 maize genomes, a total of 21,755 non-redundant insertions were identified relative to the B73 reference genome assembly out of a total of 130,026 possible non-redundant TIPs. This program provides a powerful new tool for studying genome-wide TE variation at a population level in species with large, TE rich genomes from low-cost short read resequencing data.

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## P296 @firXLLi

### Investigation of TEs DNA methylation patterns through long-read sequencing

(submitted by Xingli Li <li.14356@osu.edu>)
Full Author List: Li, Xingli[1]; Ou, Shujun[1]
[1] Department of Molecular Genetics, The Ohio State University, Columbus, Ohio, 43210

Cytosine methylation is an epigenetic DNA modification that involves the addition of a methyl group to the cytosine base. In plants, cytosine methylation occurs in CG, CHG, or CHH contexts, where H represents A, T, or C. These different contexts establish DNA methylation patterns across the genome, regulating transcriptional activities. Previous studies have revealed that CG methylation in exons positively correlated with gene expression, whereas the addition of non-CG methylation predominantly silent exons. Methylated CHH (mCHH) is relatively scarce in maize but is known to serve as boundaries between euchromatin and heterochromatin. DNA methylation plays a crucial role in silencing transposable elements (TEs), which can be lifted when plants are under abiotic stress conditions, resulting in increased TE transcriptions. However, the mechanisms by which TE methylation patterns change under abiotic stress remain largely unknown. Long-read sequencing allows precise detection of DNA methylation and overcomes mapping challenges, allowing accurate analysis of TE methylation. In this project, we identified 25% more CG methylation sites by long-read sequencing (PacBio HiFi and Oxford Nanopore) compared to short-read sequencing (EM-seq), with 93% new sites located in long terminal repeat (LTR) retrotransposons. Utilizing Nanopore sequencing, we were able to robustly profile CG, CHG, and CHH methylation patterns in maize TEs. Notably, only 5% of CG and CHG unmethylated regions (UMRs) were located within TEs, while up to 65% of mCHH islands were confined to TEs. Additionally, CG UMRs frequently co-localized with CHG UMRs or mCHH islands in TEs, a pattern different from genes. Moreover, LTR regions containing CG/CHG UMRs and mCHH islands were significantly younger than those containing only CHG UMRs or mCHH islands, suggesting transcriptional potentials. Nanopore RNA sequencing further revealed that CG/CHG UMRs, along with mCHH islands, often associate with active LTR transcriptions and marked their transcription start sites in maize. These findings suggest unique DNA methylation patterns in actively transcribed LTR retrotransposons under abiotic stress conditions.

## P297

### Investigation of the maintenance mechanism on the silenced *Ac* transposon in *id4*

(submitted by Sicong Wang <swang76@pride.hofstra.edu>)
Full Author List: Wang, Sicong[1]; Wang, Dafang[1]
[1] Hofstra University; Hempstead, NY, USA 11549

The movement of transposable elements can disrupt genes or regulatory regions, leading to mutations. To protect genome integrity, organisms have evolved effective mechanisms to silence transposons, including the initiation and maintenance of silencing across generations. In this study, we focus on the id4 silencer allele, which was previously identified as being derived from alternative transposition events. This allele synthesizes 21/22 nt and 24 nt small RNAs, which target active Ac elements to initiate silencing in the first generation. Interestingly, it has been observed that the silencing induced by the id4 allele can be maintained in subsequent generations even after the silencer itself is segregated and no longer present. While preliminary research has characterized the silencing mechanisms involved in the initiation of silencing in the presence of the id4 silencer, it has not yet explored the maintenance of silencing. This study hypothesizes that a subset of small RNAs continues to be synthesized during silencing maintenance, activating mechanisms that maintain the silencing in future generations. Our study evaluates the efficiency of silencing maintenance in subsequent generations, small RNA profiles, DNA methylation patterns, histone modifications, and transcriptional profiles in the maintained lines. The goal of this research is to deepen our understanding of transposon silencing across generations and to provide insights into how ancient silencers might maintain the heritable silencing in the maize genome.

Funding acknowledgement: National Science Foundation (NSF)

## P298

### Landscape: The evolution of transposable elements (TEs) in Maize

(submitted by Beibei Liu <beiliu@ucdavis.edu>)
Full Author List: Liu, Beibei[1]; Munasinghe, Manisha[2]; Hirsch, Candice N.[2]; Ross-Ibarra, Jeffrey[1]
[1] Department of Evolution and Ecology, University of California, Davis, CA, US, 95616
[2] Department of Agronomy and Plant Genetics, University of Minnesota, St.Paul, MN,US,55455

Although most evolutionary research has focused on one type of mutation – single nucleotide polymorphism (SNP), the transposition of transposable elements (TEs) is also a major source of mutations that can impact organismal fitness. Most work studying TEs has used methods that don't account for the mechanisms and rate of transposition, use short read-data that are inadequate to identify TEs, or focus on small genomes like Arabidopsis. Here, we develop a novel population genetic approach to study selection on TEs using the high quality long-read data from the 25 NAM lines. We identified three major factors influencing selection on TEs: 1) the distance between a TE and its nearest gene 2) the impact of TEs on nearby gene expression and 3) CG methylation induced or spread by TEs. This research is the first to use high-quality, long-read sequencing genome assemblies to investigate TE selection in a significant crop species with a typical plant genome size. Our new ΦSFS approach provides a method with higher resolution for identifying selection and could be applied to other organisms as well. Lastly, the factors identified here that influence selection on TEs deepen our understanding of the interactions and trade-offs between TEs and genes.

Funding acknowledgement: National Science Foundation (NSF)

## P299

### Mechanisms of small RNA-induced epigenetic silencing of *Ac* transposons in maize

(submitted by Dafang Wang <dafang.wang@hofstra.edu>)
Full Author List: Wang, Dafang[1]; Horowitz, Lilly[1]; Ishraq, Zuhair[1]; Huang, Charlotte[1]
[1] Hofstra University; Hempstead, NY, USA 11549

Transposable elements (TEs) are pervasive genetic elements that replicate, insert into new genomic locations, and cause mutations or chromosomal rearrangements. Small RNAs generated through an epigenetic pathway can effectively target and silence transposons. This study explores the initiation of epigenetic silencing of *Ac* transposons in maize by utilizing *Ac killer* (*Ack*), a naturally occurring silencer derived from alternative *Ac* transposition. *Ack* produces 21/22 nt and 24 nt small RNAs homologous to *Ac* sequences, enabling it to target active *Ac* elements. We investigated four maize developmental stages including germinating embryo, V2 leaf 3, V8 leaf 10, and R1 silk and observed significant enrichment of DNA methylation in all cytosine contexts, demonstrating RNA-dependent DNA methylation (RdDM). Additionally, H3K9me2 histone modifications were induced across these stages. To clarify the role of each class of small RNAs, we utilized a loss-of-function mutation in *mediator of paramutation1* (*mop1*), which encodes RDR2 in the wild type. This mutation substantially reduces the accumulation of 24 nt small RNAs, allowing us to alter the *Ack* small RNA profile. Our findings demonstrate the contributions of 21/22 nt and 24 nt small RNAs to *Ack*-induced epigenetic silencing.

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## P300

### Somatic insertional preference of the mutator transposon across distinct maize tissues

(submitted by Justin Scherer <jts34805@uga.edu>)
Full Author List: Scherer, Justin T.[1]; Nelms, Brad[1]
[1] University of Georgia, Athens, Georgia, USA, 30602

The maize Mutator (Mu) transposon is a DNA transposon with a high forward mutation rate in Mu-active lines due to its preference to insert in or near genes, typically in the 5' UTR. While we know that Mu's heritable insertion preference is centered around genes, Mu's somatic insertional preference across tissue types remains unknown. Here, we use next-generation sequencing to identify somatic Mu insertions in leaf, primary root, endosperm, and pollen. We discovered that somatic Mu insertions tended to insert near one another in clusters

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## P301

### TE-like DNA methylation in regulation of highly expressed nutrient transfer and storage genes in endosperm

(submitted by Yirui Sun <s.yirui@uga.edu>)
Full Author List: Sun, Yirui[1]; Zeng, Yibing[2]; Dawe, R. Kelly[1 2 3]; Gent, Jonathan[3]
[1] Institute of Bioinformatics, University of Georgia, Athens, GA, USA 30602
[2] Department of Genetics, University of Georgia, Athens, GA, USA 30602
[3] Department of Plant Biology, University of Georgia, Athens, GA, USA 30602

Although DNA methylation primarily represses TEs, it also represses certain genes that are methylated in sporophyte tissues but demethylated by DNA glycosylases/lyases (DNGs) in endosperm and pollen. In endosperm, demethylation specifically of maternal alleles leads to genomic imprinting of a variety of TEs and genes, including genes that encode transcription factors and other transcriptional regulators that function in early endosperm development. These imprinted DNG-target genes are characterized by TE-like methylation/demethylation in their promoters and by moderate expression levels. Here we describe a different class of DNG target genes that is characterized by methylation not only in promoters but also across the genes' coding DNA, and by unusually high expression levels. They are also unusual in their lack of introns. The majority are involved in nutrient transfer and storage, including many alpha-zein genes. These results are consistent with recent discoveries related to DNGs in pollen gene regulation and suggest a function for TE-like DNA methylation in regulation of highly expressed and highly tissue-specific genes.

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## P302

### Using parent-offspring pairs to study the inheritance patterns of *Zea mays* chromatin accessibility.

(submitted by Mark Minow <mam34190@uga.edu>)
Full Author List: Minow, Mark A.A.[1]; Marand, Alex P.[2]; Zhang, Xuan[1]; Meyer, Cullan A.[1]; Schmitz, Robert J.[1]
[1] University of Georgia, Davison Life Sciences Complex 120 E Green St., Athens, GA, USA 30602
[2] University of Michigan, Biological Sciences Building 1105 N University Ave, Ann Arbor, MI, USA 48109

Transcription factors bind specific DNA sequences, known as *cis*-regulatory elements, to regulate gene transcription. In eukaryotic genomes, the accessibility of these *cis*-regulatory elements is controlled by the chromatin environment, with accessible, nucleosome-free DNA needed for most transcription factor interactions. However, questions remain surrounding the inheritance of chromatin accessibility. To learn more about chromatin inheritance patterns, we measured seedling chromatin accessibility on a triad of inbreds (Oh43, B73 and Ki3) and their F1 progeny via Assay for Transposase Accessible Chromatin sequencing (ATAC-seq). Using a concatenated reference alignment approach, we thoroughly interrogated their accessible chromatin regions (ACRs), finding haplotype shared, unique and duplicated ACRs in these maize F1-parent plants. We categorized the mode of inheritance (additive, dominant, under/over-dominant) for ACRs genome-wide, revealing that, like genes, chromatin accessibility is largely inherited additively. Chromatin accessibility narrow sense heritability ($h^2$) estimates likewise support widespread additive ACR inheritance, and there was good concordance between high ACR heritability and previous chromatin accessibility quantitative trait loci (caQTL) detection. Narrow sense heritability was high for most accessible chromatin regions but was higher in promoters or intergenic ACRs than accessible genic regions. Indeed, transcribed ACR heritability was very similar to the gene expression heritability estimates from other groups. Finally, we exploited our parent-offspring pairs, and allele-specific reads, to find ACRs with the hallmarks of *trans* regulation – these candidate ACRs were combined with the caQTL diversity panel, empowering the detection of *trans* regulatory relationships within the maize genome.

Funding acknowledgement: National Science Foundation (NSF)

## P303

### *mop1 reshapes recombination landscapes by altering DNA methylation and chromatin states at MITEs*

(submitted by Mohammad Mahmood Hasan <m.hasanbot@gmail.com>)
Full Author List: Hasan, Mohammad Mahmood[1]; Wang, Minghui[2]; Pawlowski, Wojtek[3]; Zhao, Meixia[1]
[1] Department of Microbiology and Cell Science; University of Florida; Gainesville; FL; USA 32603
[2] Meiogenix Inc.; Center for Life Science Ventures of Cornell University; Cornell University; Ithaca; NY; USA 14850
[3] School of Integrated Plant Science; Cornell University; Ithaca; NY; USA 15853

Meiotic recombination ensures genetic diversity and proper chromosome segregation, but its regulation remains poorly understood in many species. Here, we investigate the role of Mop1 (Mediator of paramutation1), a key component of the RNA-directed DNA methylation pathway, in reshaping the recombination landscape in maize. We performed whole-genome resequencing of 97 and 110 backcrossed (BC1) individuals derived from female mop1 mutants and wild types, as well as 122 and 94 BC1 individuals derived from male mop1 mutants and wild types, identifying crossovers (COs) at high resolution. Using a Hidden Markov Model, we identified 4,048 COs, with approximately 50% occurring within a 2 kb interval across all populations. Our data reveal that mop1 has a sex-specific impact on meiotic recombination in maize, where the number of COs is significantly reduced in male mop1 mutants but remains unchanged in female mop1 mutants compared to their WT counterparts. However, in both sexes, CO numbers are reduced in the pericentromeric regions. Further analysis indicates that mop1 elevates CO frequency near miniature inverted-repeat transposable elements (MITEs). Epigenetic profiling demonstrates that mop1 significantly reduces DNA methylation and increases the abundance of open chromatin marks, such as H2A.Z and histone acetylation, at MITEs located near genes. Additionally, mop1 increases CO sites in regions of higher genetic diversity. Together, these results highlight the critical role of MOP1 in regulating meiotic recombination by modulating DNA methylation and chromatin states at transposable elements, providing new insights into the epigenetic regulation of meiotic recombination.

Gene / Gene Models described: *Mop1*; Zm00001eb080370
Funding acknowledgement: National Institutes of Health (NIH), National Science Foundation (NSF)

# Author List

# Participant list

| Participant | Organization |
|---|---|
| Aboobucker, Siddique | Iowa State University |
| Abu, Pearl | University of Ghana |
| Acosta Bayona, Jonathan | Cinvestav, Langebio |
| Adesina, Olamide | Kansas State University |
| Akozbek, Laramie | HudsonAlpha Institute for Biotechnology, Auburn University |
| Akram, Sara | Florida State University |
| Albertsen, Marc | Albertsen Crop Genetics for Humanity, LLC |
| Ali, Waqar | University of Nebraska-Lincoln |
| Anderson, Holly | University of Illinois Urbana |
| Andorf, Carson | USDA-ARS |
| Angelovici, Ruthie | University of Missouri |
| Angermeier, Ally | Donald Danforth Plant Science Center |
| Anleu Gil, Maria Ximena | University of California, Davis |
| Ansaf, Huda | University of Missouri-Columbia |
| Aragón-Raygoza, Alex | Iowa State University |
| Arora, Sofiya | University of Nebraska-Lincoln |
| Ascenzi, Robert | BASF |

| Participant | Organization |
|---|---|
| Avalos-Calleros, J.Aaron | Kansas State University |
| Awale, Prameela | University of Missouri, Columbia |
| Babiker, Ebrahiem | USDA-ARS |
| Baiochi Riboldi, Lucas | Michigan State University |
| Balint-Kurti, Peter | USDA-ARS |
| Barros da Costa Fiege, Leonardo | KWS SAAT SE & Co. KGaA |
| Baseggio, Matheus | Seneca Foods |
| Bass, Hank | Florida State University |
| Batista, Lucas | Kansas State University |
| Baxter, Ivan | Donald Danforth Plant Science Center |
| Begcy, Kevin | University of Florida |
| Bell, Harrison | Oregon State University |
| Benson, Chris | Ohio State University |
| Bernau, Vivian | USDA Plant Introduction |
| Berry, Sydney | University of Minnesota - Twin Cities |
| Berthel, Ana | Cornell University |
| Bertolini, Edoardo | Donald Danforth Plant Science Center |
| Best, Norman | USDA-ARS |

| Participant | Organization |
|---|---|
| Bhandari, Prashant | Donald Danforth Plant Science Center |
| Birchler, James | University of Missouri |
| Birnbaum, Kenneth | New York University |
| Bowser, Nathan | Iowa State University |
| Brady, Siobhan | Howard Hughes Medical Institute, University of California, Davis |
| Braud, Max | Donald Danforth Plant Science Center |
| Braun, David | University of Missouri - Columbia |
| Braun, Elliot | Michigan State University |
| Brindisi, Lara | USDA-ARS |
| Brohammer, Alex | Bayer Crop Science |
| Brottlund, Donielle | Michigan State University |
| Bryan, Kempton | University of Georgia |
| Buckler, Edward | USDA-ARS at Cornell University |
| Burges, Tom | University of Missouri |
| Burns, Michael | University of Minnesota |
| Busche, Michael | University of Wisconsin, Madison |
| Buttelmann, Gretta | Iowa State University |
| Cahn, Jonathan | CSHL |

| Participant | Organization |
|---|---|
| Caldwell, Denise | Purdue University - Botany and Plant Pathology |
| Campbell, Darwin | Iowa State University |
| Cannon, Ethy | USDA-ARS |
| Cao, Doris | University of Delaware |
| Carrillo, Rina | University of Hawaii at Manoa |
| Casto, Anna | Donald Danforth Plant Science Center |
| Chamberlain, Heather | University of Vienna |
| Char, SiNian | University of Missouri - Columbia, MO |
| Chaudhari, Vinay | Donald Danforth Plant Science Center |
| Chavez-Carranza, Mariana | Montclair State Unviersity |
| Chen, Huan | Michigan State University |
| Chen, Jeff | The University of Texas at Austin |
| Chen, Keting | Iowa State University |
| Chen, Qiuyue | University of Wisconsin-Madison |
| Chen, Szu-Ping | Cornell University |
| Chen, Yu-Ru | Iowa State University |
| Chen, Zongliang | Rutgers University |
| Cheng, Cheng | Iowa State University |

| Participant | Organization |
|---|---|
| Cheng, Xi | University of Florida |
| Cho, Michelle | Donald Danforth Plant Science Center |
| Chomet, Paul | Iowa Corn Growers/ Chomet Consulting LLC |
| Chu, Liuyang | University |
| Chumak, Nina | Uni Zurich, Dept. of Plant and Microbial Biology |
| Collier, Ray | University of Wisconsin Madison |
| Collins, Lawson | University of Wisconsin-Madison |
| Costich, Denise | Cornell University |
| Coursol, Sylvie | INRAE |
| Cryan, Elli | University of California, Davis |
| Cummings, Jordan | North Carolina State University |
| Curry, Helen Anne | Georgia Institute of Technology |
| Dallo, Maximiliano | Advanta US LLC |
| Davis, Danielle | University of Wisconsin |
| Davis, Jensina | University of Nebraska-Lincoln |
| Dawe, R. Kelly | University of Georgia |
| Dawson, Henry | Cornell University |
| Daza, Amparo | University of Wisconsin-Madison |

| Participant | Organization |
|---|---|
| de Leon Gatti, Natalia | University of Wisconsin-Madison |
| De Schepper, Veerle | Inari |
| Deb, Sontosh | North Carolina State University |
| Degenhardt, Jorg | Martin Luther University Halle |
| DeTemple, Joseph | Iowa State University |
| DeTemple, Megan | Iowa State University |
| Dewatha Pedige, Nirosha Lakmali Karunarathna | KWS SAAT SE & Co. KGaA |
| Dhakal, Rijan | Donald Danforth Plant Sciences Center |
| Dhungana, Singha | Bayer Crop Science |
| Dilkes, Brian | Purdue University |
| Dominguez, Liz | University of Illinois |
| Draves, Melissa | University of Missouri |
| Dresselhaus, Thomas | University of Regensburg |
| Drew, Makayla | University of California, Riverside |
| Du, Charles | Montclair State University |
| Du, Xuelian | University of Bonn |
| Dubois, Patrice | Limagrain |
| Edwards, Jode | USDA |

| Participant | Organization |
|---|---|
| Egger, Rachel | Syngenta |
| El-Walid, Mohamed | Cornell University |
| Ensor, Maya | University of Missouri-Columbia |
| Evans, Lukas | Cornell University |
| Eveland, Andrea | Donald Danforth Plant Science Center |
| Farmer, Erin | Cornell University |
| Faustinoni Bruno, Maria Helena | UNICAMP |
| Fernandez, Edward Cedrick | North Dakota State University |
| Ferris, Tyler | University of Nebraska-Lincoln |
| FInnegan, Colin | Iowa State University |
| Fleming, Zachary | University of Nebraska Lincoln |
| Flint-Garcia, Sherry | USDA-ARS Columbia, MO |
| Fowler, John | BPP Dept, Oregon State University |
| Francis, Nicholas | Oregon State University |
| Fritz, Katelyn | North Carolina State University |
| Gage, Joseph | NCSU |
| Gaisie, Maa Ahema | Nelms Lab at University of Georgia |
| Gallavotti, Andrea | Waksman Institute, Rutgers University |

| Participant | Organization |
|---|---|
| Gao, Huirong | Corteva Agriscience |
| Gao, Xiquan | State Key Laboratory of Crop Genetics & Germplasm Enhancement and Utilization, Nanjing Agricultural University |
| Garberding, Andrea | University of Wisconsin Madison |
| Gardiner, Jack | University of Missouri-Retired |
| Gardner, Patrick | Montclair State University |
| Garrido, Rommel Jr. | University of Minnesota-Twin Cities |
| Geadelmann, Lander | Iowa State University |
| Gent, Jonathan | University of Georgia |
| German, Liam | Crop Science Centre, University of Cambridge |
| Ghenov, Fernanda | University of Hawai'i at Manoa |
| Gilbert, Nathan | University of Missouri - Columbia |
| Glover, Zehta | North Carolina State University |
| Goldberg, Erik | Michigan State University |
| Gómez-Trejo, Libia F. | University of Nebraska-Lincoln |
| Gonzalez, Gerardo | University of Hawaii at Manoa |
| Gooden, Caleb | School: The Ohio State University |
| Gorman, Zachary | USDA-ARS |

| Participant | Organization |
|---|---|
| Gregory, Luke | Cornell University |
| Griffin, Brianna | Iowa State University |
| Grossniklaus, Ueli | University of Zurich |
| Grove, Ryleigh | University of Nebraska-Lincoln |
| Grover, Corrinne | Iowa State University |
| Gruening, Vencke | Iowa State University |
| Gualtieri, Ruggero | Alma Mater Studiorum Università di Bologna |
| Gustin, Jeffery | Maize Genetics COOP Stock Center |
| Guthrie, Katy | University of Minnesota |
| Haagensen, Sora | Brandeis University |
| Hagedorn, Kara | Schnable Lab |
| Hale, Charlie | Cornell University |
| Haley, Olivia | ORISE |
| Hammett, Cole | North Carolina State University |
| Hannah, Curt | University of Florida |
| Hansen, Eric | Iowa State University |
| Harper, Riley | University of Florida |
| Hasan, Mohammad Mahmood | University of Florida |

| Participant | Organization |
|---|---|
| Hava, Kevin | Donald Danforth Plant Science Center |
| Hayford, Rita | MaizeGDB |
| Hein, Kirsten | Donald Danforth Plant Science Center |
| Helm, Matt | USDA-ARS |
| Henry, Caroline | Danforth Plant Science Center |
| Herr, Samuel | |
| Hinrichsen, Jake | Iowa State University |
| Hirsch, Candice | University of Minnesota |
| Hochholdinger, Frank | University of Bonn |
| Hodge, John | University of Illinois |
| Hodge, John | University of Illinois |
| Holding, David | University Of Nebraska |
| Holland, Jim | USDA-ARS |
| Hostetler, Ashley | University of Delaware |
| Hsu, Sheng-Kai | Cornell University |
| Hua, Jian | Cornell University |
| Hufford, Matthew | Iowa State University |
| Hunter, Charles | USDA-ARS |

| Participant | Organization |
|---|---|
| Ikiriko, Irene | University of Delaware |
| Insko, Lauren | North Carolina State University |
| Isles, Taylor | University of Missouri-Columbia |
| Jackson, Dave | Cold Spring Harbor Laboratory |
| Jaiswal, Pankaj | Oregon State University / National Science Foundation |
| Jamann, Tiffany | University of Illinois |
| Janik, Magdalena | Donald Danforth Plant Science Center |
| Jayasinghe, Vishadinie | Iowa State University |
| Jeffers, Daniel | USDA-ARS |
| Jensen, Sarah | Syngenta |
| Ji, Zhongjie | University of Nebraska-Lincoln |
| Jiang, Hui | Donald Danforth Plant Science Center |
| Johnson, Quinn | Cornell University |
| Jones, Todd | Corteva agriscience |
| Julius, Benjamin | Bayer Crop Science |
| Justen, Veronica | UW-River Falls |
| K C, Shreejana | University of Delaware |
| Kaeppler, Shawn | University of Wisconsin-Madison |

| Participant | Organization |
|---|---|
| Kamei, Thanduanlung | University of Delaware |
| Kang, Minjeong | Ohalo Genetics |
| Kappelmann, Kiara | Iowa State University |
| Kasule, Faizo | Iowa State University of Science and Technology |
| Kaur, Amanpreet | Purdue University |
| Kaur, Shivreet | North Carolina State University |
| Kelleher, Micah | Donald Danforth Plant Science Center |
| Kellogg, Elizabeth | Donald Danforth Plant Science Center |
| Kelly, Jacob | University of Missouri |
| Kenchanmane Raju, Sunil | University of California Riverside |
| Kermani, Taran | University of Delaware |
| Khalid, Iman | University of Florida |
| Khangura, Rajdeep | Purdue University |
| Kick, Daniel | USDA-ARS |
| Kim, Bitgoeul | Iowa State University |
| Kim, Jeonghwa | University of Delaware |
| Kim, Saet-Byul | University of Nebraska-Lincoln |
| Klaas, Emma | University of Missouri |

| Participant | Organization |
|---|---|
| Koch, Karen | University of Florida |
| Koltun, Alessandra | Corteva Agriscience |
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| Kriz, Alan | Retired |
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| Kumar, Rohit | Clemson University |
| Kunduru, Bharath | Clemson University |
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| LaFond, Harper | USDA-ARS |
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| Lama, Sunita | Donald Danforth Plant Science Center |
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| Lane, Madison | Iowa State University |
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| Larsson, Sara | Corteva Agriscience |
| Larue, Huachun | BAYER |
| Laseter, Jesse | Donald Danforth Plant Science Center |

| Participant | Organization |
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| Linders, Kyle | University of Nebraska-Lincoln |
| Lipka, Alexander | Univeristy of Illinois |
| Lipps, Sarah | University of Illinois, Urbana-Champaign |

| Participant | Organization |
|---|---|
| Lisch, Damon | Purdue University |
| Liu, Alexander | Donald Danforth Plant Science Center |
| Liu, Beibei | University of California, Davis |
| Liu, Hua | University of Missouri |
| Liu, Hui | Kansas State University |
| Liu, Huyu | Iowa State University |
| Liu, Sanzhen | Kansas State University |
| Liu, Wen-Yu | NCSU |
| Liu, Xuan | Iowa State University |
| Liu, Yue | Iowa State University |
| Liu, Zong-Yan | Cornell University |
| Lopez-Corona, Lina | ORISE |
| Lu, Jiawen | Iowa State University |
| Lu, Zhenyuan | Cold Spring Harbor Laboratory |
| Lubberstedt, Thomas | Iowa State University |
| Lubkowitz, Mark | Saint Michael's College |
| Luebbert, Collin | Donald Danforth Plant Science Center |
| Luo, Yun | Cornell University |

| Participant | Organization |
|---|---|
| Lynn, Jason | Cold Spring Harbor Laboratory |
| Lyudmila, Lyudmila | Corteva Agriscience |
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| Mackenzie, Sally | EpiCrop Technologies |
| Madzima, Thelma | Michigan State University |
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| Mascarenhas, Jane | Kansas State University |
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| McCargar, Desere' | Iowa State University |
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| Participant | Organization |
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| Medina Montes, Maria Camila | Oregon State University |
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| Moran Lauter, Adrienne | USDA-ARS-CICGRU |
| Morton, Kyla | EpiCrop Technologies |

| Participant | Organization |
|---|---|
| Mourad, Nadia | University of Florida |
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| Mu, Qi | University of Delaware |
| Mueller, Sebastian | Oregon State University |
| Mullens, Alexander | University of Illinois |
| Muluya, Solomon Ndolo | Other |
| Munasinghe, Manisha | University of Minnesota Twin Cities |
| Murphy, Katherine | Donald Danforth Plant Science Center |
| Muszynski, Michael | University of Hawaii - Manoa |
| Navrude, Spencer | Kansas State University |
| Negus, Karlene | Iowa State University |
| Nelms, Brad | University of Georgia |
| Newton, Kathleen | University of Missouri |
| Niyorukundo, Jonathan | University of Nebraska Lincoln |
| O'Connor, Lily | Donald Danforth Plant Science Center |
| Ohlson, Erik | USDA-ARS, Wooster OH |
| Ojeda Rivera, Jonathan | Cornell University |
| Okamuro, Diane | National Science Foundation |

| Participant | Organization |
|---|---|
| Oliver, Sarah | University of Missouri - Columbia |
| Olomukoro, Enameguono | Purdue University |
| Olson, Jacob | Purdue University |
| Ou, Shujun | The Ohio State University |
| Oyiga, Benedict Chijioke | KWS SAAT SE & Co. KGaA |
| Park, Tae-Chun | Iowa State University |
| Pawlowski, Wojtek | Cornell University |
| Perez, Veronica | Cornell University |
| Pierce, Emilia | University of Delaware |
| Pil, Hannah | North Carolina State University |
| Pinheiro, Ana Lúcia | University of Campinas |
| Pitz, Marion | University of Bonn |
| Pollpeter, Amy | Iowa State University |
| Portwood, John | USDA-ARS |
| Price, Colin | MRIGlobal |
| Quinones, Alejandra | University of Minnesota |
| Ragas, Richie Eve | Cornell University |
| Rai, Maruti Nandan | University of Illinois |

| Participant | Organization |
|---|---|
| Raj, Aditya | Iowa State University |
| Rajagopal, Meera | The Ohio State University |
| Rasmussen, Carolyn | University of California, Riverside |
| Rellan Alvarez, Ruben | North Carolina State University |
| Resende, Marcio | University of Florida |
| Rodene, Eric | University of Nebraska-Lincoln |
| Rodriguez-Zapata, Fausto | North Carolina State University |
| Rodriguez, Carmen | The Ohio State University |
| Rogers, Tess | Donald Danforth Plant Science Center |
| Romano, Joseph | University of Delaware |
| Romay Alvarez, Maria Cinta | Cornell University |
| Ronceret, Arnaud | UNAM |
| Ross-Ibarra, Jeffrey | UC Davis |
| Ruban, Alevtina | KWS SAAT SE Co. & KGaA |
| Ruggiero, Diana | Oregon State University |
| Runyon, Matthew | University of Illinois Urbana-Champaign |
| Sachs, Marty | Maize Genetics COOP Stock Center |
| Sagae, Vitor | University of Florida |

| Participant | Organization |
|---|---|
| Salazar Vidal, Miriam Nancy | University of Missouri |
| Sama, Andrea | University of California, San Diego |
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| Sandhu, Jaspreet | Donald Danforth Plant Science Center |
| Sangjan, Worasit | USDA-ARS |
| Sangra, Ankush | University of Georgia |
| Sapkota, Dikshya | University of Illinois Urbana Champaign |
| Scanlon, Michael | Cornell University |
| Scherer, Justin | University of Georgia, Athens |
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| Schulz, Aimee | University of Minnesota |
| Schumacher, Ally | Michigan State University |
| Scott, Blake | University of Missouri |
| Scott, Paul | USDA-ARS-CICGRU |
| Scroggs, Taylor | University of Georgia |
| Searl, John | University of Wisconsin-Madison |

| Participant | Organization |
|---|---|
| Seetharam, Arun | Purdue University |
| Seigel, Eliza | Donald Danforth Plant Science Center |
| Shanmugaraj, Nandhakumar | Cold Spring Harbor Laboratory (CSHL), NY, USA |
| Sharma, Malika | University of Missouri- Columbia |
| Shi, Xiaowen | Zhejiang University |
| Shrestha, Nikee | University of Nebraska-Lincoln |
| Sigmon, Brandi | University of Nebraska-Lincoln |
| Singh, Anuradha | Michigan State University |
| Singh, Deepjyoti | North Carolina State University |
| Singh, Jasnoor | University of Missouri-Columbia |
| Singh, Raksha | USDA-ARS |
| Slotkin, R. Keith | Donald Danforth Plant Science Center |
| Snodgrass, Samantha | University of California, Davis |
| Soenksen, Chad | Colorado State University |
| Somers, Julian | University of Georgia |
| Sorensen, Peyton | University of Illinois Urbana-Champaign |
| Sosso, Davide | Heritable Agriculture |
| Sparks, Erin | University of Delaware; University of Missouri; Donald Danforth Plant Science Center |

| Participant | Organization |
|---|---|
| Stam, Maike | University of Amsterdam |
| Stitzer, Michelle | Cornell University |
| Strable, Josh | Iowa State University |
| Studer, Anthony | University of Illinois |
| Subedi, Manoj | Univeristy of Delaware |
| Sullivan, Madsen | Conagra Brands |
| Sun, Yirui | University of Georgia |
| Swentowsky, Kyle | Cold Spring Harbor Laboratory |
| Sylvester, Anne | University of Chicago - MBL |
| Tammineni, Lakshmipriyanka | Iowa State University |
| Tang, Yuan | China Agricultural University |
| Thies, August | Donald Danforth Plant Science Center & University of Missouri |
| Thomas, Ty | North Carolina State University |
| Thompson, Addie | Michigan State University |
| Thompson, Beth | East Carolina University |
| Tibbs-Cortes, Laura | USDA-ARS, MaizeGDB |
| Tong, Jingjing | University of Missouri |
| Topp, Christopher | Donald Danforth Plant Science Center |

| Participant | Organization |
|---|---|
| Torres-Rodríguez, Vladimir | University of Nebraska-Lincoln |
| Tracy, William | University of Wisconsin-Madison |
| Tran, Alexandria | University of Illinois: Urbana-Champaign |
| Tran, Thu | Cold Spring Harbor Laboratory |
| Traylor, Zachary | University of Missouri - Columbia |
| Tripka, Abigail | University of California San Diego |
| Tuberosa, Roberto | University of Bologna |
| Turkus, Jonathan | University of Nebraska-Lincoln |
| Twohey III, Robert | University of Illinois |
| Ullagaddi, Chidanand | University of Nebraska-Lincoln |
| Uy, Jaclyn Nicole | University of Hawaii |
| VanBuren, Robert | Michigan State University |
| Vanous, Adam | USDA Plant Introduction |
| Vincent, Grace | North Carolina State University |
| Vollbrecht, Erik | Iowa State University |
| Wagner, Ruth | Bayer Crop Science |
| Walberg, Noah | Iowa State University |
| Walbot, Virginia | Stanford University |

| Participant | Organization |
|---|---|
| Walter, Nathalie | University of Wisconsin-Madison |
| Wang, Bingxin Wang | Iowa state university |
| Wang, Dafang | Hofstra University |
| Wang, Kan | Iowa State University |
| Wang, Mingyu | University of Georgia |
| Wang, Shuya | University of Illinois at Urbana- Champaign |
| Wang, Sicong | Hofstra University |
| Ware, Doreen | USDA ARS @ Cold Spring Harbor Laboratory |
| Washburn, Jacob | USDA-ARS |
| Webster, Brandon | Michigan State University |
| Weil, Clifford | National Science Foundation |
| Weldt, Carolina | North Carolina State University |
| Wendel, Jonathan | Iowa State University, Ecology, Evolution, and Organismal Biology Department |
| Wendt, Matthew | Iowa State University |
| Wheeler, Emily | NC State University |
| Whipple, Clinton | Brigham Young University |
| Whitfield, Anna | North Carolina State University |
| Wodehouse, Heather | University of Wisconsin - Madison |

| Participant | Organization |
|---|---|
| Woodhouse, Margaret | USDA-ARS |
| Wu, Wei | Molbreeding Biotechnology |
| Würstl, Lukas | Technical University Munich |
| Xavier, Cesar | North Carolina State University |
| Xiang, Zhaocheng | University of Nebraska-Lincoln |
| Xiao, Yuguo | Donald Danforth Plant Science Center |
| Xu, Fang | Shandong University |
| Xu, Gen | University of Nebraska-Lincoln |
| Xu, Wenwei | Texas A&M University |
| Xu, Xiaosa | University of California, Davis |
| Yandeau-Nelson, Marna | Iowa State University |
| Yang, Bing | University of Missouri |
| Yang, Hailong | University of Massachusetts Amherst |
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| Yang, Jinliang | University of Nebraska-Lincoln |
| Yang, Qin | Northwest A&F University |
| Yassitepe, Juliana | Embrapa |
| Yavuz, Caner | University of Nebraska-Lincoln |

| Participant | Organization |
|---|---|
| Yeboah, Akwasi | University of Florida |
| Yu, Jianming | Iowa State University |
| Yu, Xingyao | University of California, Davis |
| Zebosi, Brian | Oregon state University |
| Zelkowski, Mateusz | Cornell University |
| Zhai, Jingjing | Cornell University |
| zhang, ruqiang | Cornell University |
| Zhang, Zhengzhi | University of Missouri |
| Zhao, Meixia | University of Florida |
| Zheng, Xixi | University of Regensburg |
| Zhong, Tao | NC State University |
| Zhou, Jiaqi | Cold Spring Harbor Laboratory |
| Zhou, Yaping | University of Bonn |
| Ziegler, Greg | Donald Danforth Plant Science Center |
| Zimina, Olga | Cornell University |
| Zimmerman, David | Cold Spring Harbor Laboratory |

# History of the Maize Genetics Conference

| Year | Annual | Location | Dates | Chair |
|---|---|---|---|---|
| 2025 | 67 | St. Louis, Missouri | March 6-9 | Sherry Flint-Garcia |
| 2024 | 66 | Raleigh, North Carolina | February 29 - March 3 | Rubén Rellán-Álvarez |
| 2023 | 65 | St. Louis, Missouri | March 16-19 | Matthew Hufford |
| 2022 | 64 | St. Louis, Missouri | March 31 - April 3 | Erin Sparks |
| 2021 | 63 | Online | March 8-12 | Marna Yandeau-Nelson |
| 2020 | 62 | Online | June 25-26 | Clinton Whipple |
| 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 |

| Year | Annual | Location | Dates | Chair |
|---|---|---|---|---|
| 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 |
| 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

# Notes

# Notes

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