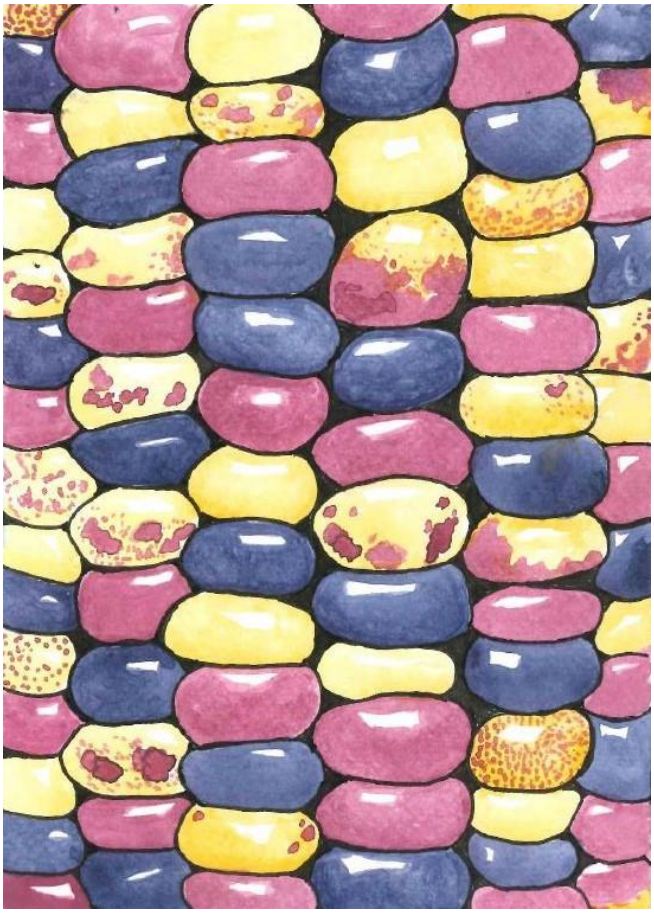
66th Annual Maize Genetics Meeting

Program and Abstracts

February 29 – March 3, 2024



MGC

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This conference received financial support from:

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

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Cover image description

Watercolor corn, mimicking Barbara McClintock's.

Cover art by

Sarah Oliver
University of Missouri
USA

General Information

Meeting Registration

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

Meals

All meals will be served in Ballrooms B&C; 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 Ballroom A.

Posters will be presented in Ballrooms B&C, adjacent to where the meals will be held. Posters should be hung on Thursday at 3 PM and stay up until Sunday morning but must be removed by 9 AM 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The maize meeting is a forum for presenting and discussing unpublished material. **Photographing or recording of talks and posters is <u>not</u> 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 2024 Maize Genetics Meeting.

All attendees of the MGM in-person meeting are encouraged to be fully vaccinated against COVID-19 and up to date on their flu shots before attending the conference.

You **may NOT** attend the conference if you:

a. Are currently required to be in isolation for COVID-19.
b. Are sick and suspect that you have COVID-19 or the flu.
c. Have had a fever within the past 24 hours.

Upon picking up your badge at the in-person conference, we will ask you to certify that you have met the above requirements. If we find that you have knowingly falsified this information, you will forfeit your membership to the Maize Genetics Cooperation and be expelled from the meeting with no refunds.

Masks are encouraged when in common spaces but not required. Attendees wearing masks are encouraged to wear the most protective mask possible such as N95, KN95, or at a minimum surgical masks, while at the MGM to limit the spread of disease.

If you develop COVID-19 symptoms at the Maize Meeting, please stay in your hotel room, and follow CDC guidance, and if you need assistance, please contact the hotel front desk or Tricia Simmons at 720-250-7033.

The MGC has approved these enhanced health and safety measures to protect you, other attendees, and hotel and conference staff. All attendees must abide by these guidelines and follow instructions posted on-site. The MGC assumes no responsibility for liability or financial hardship that may arise during or as a result of your attendance at the meeting. This includes but is not limited to, liability arising from illness, injury, or death associated with infection of COVID-19, flu, communicable disease or complications, symptoms or other effects resulting from contracting COVID-19, the flu, or other communicable diseases.

Hospitality

After the evening sessions on Thursday and Friday there will be informal socializing and poster gazing in Ballrooms B&C, with refreshments, games, and open bar provided from 9 PM - 12 AM. On Saturday evening there will be informal socializing in the Ballrooms B&C, with refreshments and cash bar from 9 PM - Midnight, trivia from 10 PM - 12 AM.

Access to recorded sessions

All talks and sessions will be recorded and 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 2024 Steering Committee

Rubén Rellán-Álvarez, Chair	(rrellan@ncsu.edu)
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Ex officio:
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Erin Sparks - Treasurer
Darwin Campbell – Planning / Audio Visual
Marty Sachs - Guru
John Portwood - Logistics Coordinator

Meeting planning:
Tricia Simmons – Conference Direct
Garrett Simmons – Conference Direct

Acknowledgments

Many thanks go to Carson Andorf, 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, 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 Raleigh Convention Center 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 66th Maize Genetics Meeting.

From the Maize Genetics Cooperation Board of Directors

Maize Genetics Awards:

The 2024 MGC Cooperator Awardees

Carson Andorf, USDA-ARS
Darwin Campbell, Iowa State University
John Portwood, USDA-ARS

The 2024 MGC Leadership Awardees

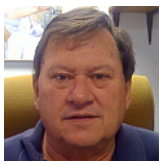
Mark Lubkowitz, Saint Michael's College
Ruth Wagner, Bayer Crop Science

The 2024 M. Rhoades Early Career Awardee

Rubén Rellán Álvarez, North Carolina State University

The 2024 L. Stadler Mid-Career Awardee

Jinsheng Lai, China Agricultural University



The 2024 R. Emerson Lifetime Awardees

Vicki Chandler, Minerva University
L. Curt Hannah, University of Florida
Jesús Sánchez González, Universidad de Guadalajara

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 **2024** Barbara McClintock Prize for Plant Genetics and Genome Studies has been awarded to Dr. Caroline Dean who will present a McClintock Prize Address on Friday, March 1, 11:40am EST (See Page 29).

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

Support from the National Science Foundation

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 the Maize Genetics Network Enhancement via Travel (MaGNET), Enhancing Institutional Access (EIA), and Disciplinary Breadth (DB) award programs. These programs broaden the participation of historically marginalized groups with an interest in exploring research possibilities in maize genetics by providing funding to attend the meeting. These 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.

The MaGNET Program and 2024 Awards

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

Each MaGNET Award helps defray the cost of attending the Maize Genetics Meeting, including registration, and for in person-meetings- food, lodging and airfare. In addition, each awardee is paired with an experienced 'Maize Mentor', who will help the awardee navigate the conference. Awardees are identifiable by a special notation on their name tags, and many of them are attending the MGM for the first time – please congratulate these scientists and welcome them to our famously hospitable conference!

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

2024 MaGNET Awardees

Undergraduate

Ramsey Chaaban, Davidson College ..... Poster #314
Theresa Clark, Cold Spring Harbor Laboratory ..... Poster #105
Gerardo Gonzalez, University of Hawaii ..... Poster #15
Daniela Meson de la Fuente, Oakland University ..... Poster #81
Hunti Xiong, Hamline University ..... Poster #6

Graduate Student

Clarice Gonzales, Iowa State University ..... Poster #71
Taylor Isles, Montclair State University ..... Poster #285
Samantha Jean, Montclair State University ..... Poster #284

The MaGNET program of the Maize Genetics Meeting is supported by grant IOS-2329928 from the National Science Foundation.

Expanding Institutional Access and Disciplinary Breadth Awards

Expanding Institutional Access (EIA) and Disciplinary Breadth (DB) are two financial aid programs that seek to recruit and retain scientists from diverse institutions and plant-related disciplines into the maize research community by encouraging their attendance at the Annual Maize Genetics Meeting (MGM). The EIA program seeks to welcome scientists from diverse institutions including community colleges, PUIs, and masters granting institutions into the maize community by encouraging their attendance at the MGM. The Disciplinary Breadth (DB) program seeks to recruit and retain scientists (advanced graduate students, post-docs, and early-career faculty) from plant-related disciplines into the maize research community. The DB program has recently been expanded to support attendance at the meeting for graduate students and postdocs from historically underrepresented groups, regardless of discipline. Both programs provide an opportunity for researchers from diverse disciplines that have potential to enrich the maize community to learn about maize genetics by connecting with scientists in the maize genetics community, exploring potential collaborations, and developing career contacts.

Each award helps defray the cost of attending the Maize Genetics Meeting, including registration, and for in person-meetings- food, lodging and airfare. Awardees are identifiable by a special notation in their badges, and many of them are attending the MGM for the first time – please congratulate these scientists and welcome them to our famously hospitable conference!

All applicants must show strong potential for a career in the biological sciences, and be either citizens or permanent residents of the USA; or employed at a US-based institution. To help provide a more integrative and effective experience at the Meeting for student awardees, faculty who accompany one or more eligible student applicants are also eligible to apply for a EIA or DB award.

2024 EIA Awardees

Student

Emilie Hoffmann, Saint Michael's College

Emma Klaas, Truman State University ..... Poster #117

2024 DB Awardees

Student

Denise Caldwell, Purdue University ..... Poster #121

Jieli Wegerif, University of Florida..... Poster #274

The EIA and DB programs of the Maize Genetics Meeting are supported by grant IOS-2329928 from the National Science Foundation.

Broadening International Participation Awards

The 2024 Broadening International Participation Award program seeks to promote international attendance for researchers from countries that are historically under-represented at the Maize Meeting. This 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.

Graduate Students
Abolade Lawrence Olanrewaju, University of Ibadan, Nigeria

Postdoctoral Researchers
Priya Garkoti, G.B. Pant University of Agriculture & Technology, India
Aysha Jameel, Biological Research Centre Szeged, Hungary
Bikkasani Mythri, Punjab Agricultural University, India

Research Scientists
Mohammad Ismail, Agricultural Research Center, Egypt

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 2023, 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:

- 104 genomes
- Over 2 million gene model annotations
- Pan-gene dataset, which includes 58 annotations
- Over 500 downloadable files
- 351 target databases in BLAST
- Genome browsers for select quality genomes with over 1,500 total tracks
- 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
- PanEffect, a tool for exploring variant effects.
- AlphaFold and ESMFold protein structures on the browser and gene model pages
- Protein structure search and comparison tools**
- 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

*Woodhouse MR et al. (2021) A pan-genomic approach to genome databases using maize as a model system. BMC Plant Biology. doi: https://doi.org/10.1186/s12870-021-03173-5.

**Woodhouse MR et al. (2023) Maize Protein Structure Resources at the Maize Genetics and Genomics Database. Genetics. doi: https://doi.org/10.1093/genetics/iyad016.

Thank you to the 2023 MaizeGDB Editorial Board Members!
Anuradha Singh, Postdoc, Michigan State University (2nd Year!)
Qiang Ning, Huazhong Agricultural University, China
Aimee Uyehara, University of California-Riverside
Keting Chen, Iowa State University
Jan Yun, Chinese Agricultural University, China

Editorial Board DEI Papers recommended by CODIE Editors!
Andrew Egesa, Graduate Student, University of Florida, Gainesville, FL

MaizeGDB has partnered with *microPublication Biology!*

***microPublication Biology* (Caltech Publishers) is a new 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 $250.

Here are some recent maize publications:

Lucas JR, Dupree B. Stomatal pore width and area measurements in Zea mays. MicroPubl Biol. 2023 Aug 4;2023:10.17912/micropub.biology.000893. doi: 10.17912/micropub.biology.000893.

Hazelwood OS, Hostetler AN, Ikiriko II, Sparks EE. Characterization of mechanosensitive MSL gene family expression in Zea mays aerial and subterranean brace roots. MicroPubl Biol. 2023 Jun 16;2023:10.17912/micropub.biology.000759. doi: 10.17912/micropub.biology.000759.

Siosiou A, Sparks EE, Tsialtas IT. Brace roots in C 3 Poaceae: where have they gone? MicroPubl Biol. 2023 Sep 20;2023:10.17912/micropub.biology.000939. doi: 10.17912/micropub.biology.000939.

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 Ballroom A
Posters will be displayed in the Ballroom B&C

Wednesday, February 28, 2024

8:00 AM – 10:00 PM	**OPTIONAL PRE-CONFERENCE WORKSHOPS**	
8:00 AM - 12:00 PM	**Maize Crop Germplasm Committee**	Room 302C
1:00 PM – 6:00 PM	**Corn Breeding Research**	Room 302A/B
7:00 PM – 10:00 PM	**Genetics of Maize-Microbe Interactions**	Room 301A

Thursday, February 29, 2024

8:00 AM – 6:00 PM	**OPTIONAL PRE-CONFERENCE WORKSHOPS**	
8:00 AM – 5:00 PM	**Corn Breeding Research**	Rooms 302A/B
8:30 AM - 5:30 PM	**Genetics of Maize-Microbe Interactions Workshop**	Room 301A
9:00 AM - 4:00 PM	**Maize Development and Cell Biology Workshop**	Room 301B
3:00 PM - 4:00 PM	**Gramene Workshop**	Room 304
4:00 PM - 5:30 PM	**MaizeGDB: Pan-genome and AI resources**	Room 304
3:00 PM – 9:30 PM	**REGISTRATION** (Registration Lobby)	
3:00 PM – 6:00 PM	**POSTER HANGING** (Ballrooms B&C)	
5:00 PM – 5:45 PM	**MaGNET Awardees and Mentors Introductions**	Room 302B
6:00 PM – 7:00 PM	**DINNER** (Ballrooms B&C)	

Thursday, February 29, 2024 (continued)

7:00 PM – 9:00 PM	**SESSION 1 – WELCOME / FOSTERING DIVERSITY IN THE MAIZE COMMUNITY AND KEYNOTE SPEAKER** Chair: Rubén Rellán Álvarez	Pages 24 & 25.
7:00 PM	**WELCOME AND ANNOUNCEMENTS**	(Ballroom A)
7:20 PM	**Ricardo Salvador, Union of Concerned Scientists** *Phenomenology for maize experts: what do you see when you contemplate the map of global maize production?*	[KS1]
8:10 PM	**Peter Balint-Kurti, Agriculture Research Service, USDA** *Selected highlights of maize disease resistance genetics research: These are a few of my favorite things.*	[KS2]
9:00 PM – 12:00 AM	**INFORMAL POSTER VIEWING & HOSPITALITY**	(Ballroom B&C)

Friday, March 1, 2024

7:00 AM – 7:50 AM	**BREAKFAST** (Ballrooms B&C)	
7:30 AM – 12:30 PM	**REGISTRATION** (Registration Lobby)	
8:00 AM – 10:10 AM	**SESSION 2 – THE GENES THAT MAKE MAIZE** Chair: Madelaine Bartlett	Talks 1-5. Pages 30-34.
8:00 AM	**ANNOUNCEMENTS**	(Ballroom A)
8:00 AM	**Xiquan Gao, Nanjing Agricultural University** *An exocyst component ZmEXO70F4 coordinates with jasmonate signaling pathway to regulate maize immunity to Gibberella stalk rot*	[T1]
8:20 AM	**Annis Richardson, University of Edinburgh** *Crazy leaves and truffula trees*	[T2]
8:40 AM	**Yun Luo, Huazhong Agricultural University** *A thiamin pyrophosphate kinase encoding gene ZmTDPK2 finely regulates ear length and grain yield in maize*	[T3]
9:00 AM	**George Chuck, University of California Berkeley** *A novel domestication gene establishes developmental boundaries*	[T4]
9:20 AM	**Mark Lubkowitz, Saint Michael's College** *Leaf angle and curricular innovation: a CURE for research and undergraduate education*	[T5]
9:40 AM	**Poster Lightning Talks**	
10:00 AM – 10:30 AM	**BREAK**	**Ballroom ABC Lobby**
10:30 AM – 12:30 PM	**SESSION 3 – KEYNOTE SPEAKER AND McCLINTOCK PRIZE PRESENTATION** Chair: Petra Wolters	Pages 26 & 29.
10:30 AM	Introduction	(Ballroom A)
10:40 AM	**Terri Long, North Carolina State University** *From the stars to your table - plants as complex conduits for iron nutrition*	[KS3]
11:30 AM	**Mark Lubkowitz, Saint Michael's College** *McClintock Prize Presentation Introduction*	
11:40 AM	**Caroline Dean, John Innes Center** *Polycomb memory to register environmental exposure*	[M1]

Friday, March 1, 2024 (continued)

12:30 PM – 1:30 PM	**LUNCH** (Ballrooms B&C) MaGNET/PUI Networking Lunch MGC BoD and MGAC Lunch	 (301A) (301B)
1:30 PM – 4:30 PM	**POSTER SESSION 1** (Ballrooms B&C)	
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 Ballrooms B&C

4:40 PM – 6:00 PM	**SESSION 4 – NEW TOOLS AND RESOURCES I** Chair: Lander Geadelmann	 Talks 6-8. Pages 35-37.
4:40 PM	**Jonathan Matheka, University of Wisconsin-Madison** *Optimized ZmWOX2A-based transformation system enhances somatic embryogenesis, transformation efficiency, and gene editing efficiency in maize (Zea mays L.) and sorghum (Sorghum bicolor L.)*	[T6]
5:00 PM	**Steve Johnson, Bayer** *Harnessing AI for Agricultural Innovation*	[T7]
5:20 PM	**Micah, Kelleher, Donald Danforth Plant Science Center** *A practical haplotype graph map of the ZeaSynthetic population enables integration of teosinte alleles into breeding efforts*	[T8]
5:40 PM	**Poster Lightning Talks**	
6:00 PM – 7:00 PM	**DINNER** (Ballrooms B&C) Bayer Student/Postdoc Dinner	 (301A)

Friday, March 1, 2024 (continued)

7:00 PM –9:00 PM	**SESSION 5 – NEW TOOLS AND RESOURCES II** Chair: Graziana Taramino **AWARDS**	Talks 9-10. Pages 38-39.
7:00 PM	**Esteban Bortiri, Syngenta** *Cyto-swapping in maize by haploid induction with a cenh3 mutant*	[T9]
7:20 PM	**Javier Mendoza Revilla, InstaDeep** *A foundational large language model for edible plant genomes provides accurate predictions of regulatory elements and gene expression in the maize genome*	[T10]
7:40 PM	**Poster Lightning Talks**	
8:00 PM	**John Fowler, Oregon State University** *Presenting: Cooperator and Leadership Awards*	
8:20 PM	**Andrea Eveland, Donald Danforth Plant Science Center** *Presenting: M. Rhoades Early-Career, L. Stadler Mid-Career*	
8:40 PM	**Marna Yandeau-Nelson, Iowa State University** *Presenting: R. Emerson Lifetime Awards*	
9:00 PM – 12:00 AM	**INFORMAL POSTER VIEWING & HOSPITALITY**	(Ballrooms B&C)

Saturday, March 2, 2024

7:00 AM – 8:00 AM	**BREAKFAST** (Ballrooms B&C)
8:00 AM – 12:00 PM	**REGISTRATION** (Registration Lobby)
8:00 AM – 10:00 AM	**SESSION 6 – THE NITROGEN CONUNDRUM** Chair: Frank Hochholdinger Talks 11-15. Pages 40-44.
8:00 AM	**Jean-Michel Ane, University of Wisconsin-Madison** [T11] *Biological nitrogen fixation on the aerial roots of maize for sustainable agriculture*
8:20 AM	**Janeen Braynen, Cold Spring Harbor Laboratory** [T12] *Decoding nitrogen use efficiency in maize and sorghum: Insights from comparative gene regulatory networks for sustainable agriculture*
8:40 AM	**Sheng-Kai Hsu, Cornell University** [T13] *The nitrogen cycle in the rhizosphere of diverse grass species in Andropogoneae*
9:00 AM	**Stephanie Klein, Iowa State University** [T14] *Genomic structural variation underlies differential root responses to nitrogen stress in maize*
9:20 AM	**Angela Kent, University of Illinois** [T15] *Mining ancient genomes for microbiome associated phenotypes for nutrient retention in agroecosystems*
9:40 AM	**Poster Lightning Talks**
10:00 AM – 10:30 AM	**BREAK** Ballroom ABC Lobby
10:30 AM – 12:30 PM	**SESSION 7 – KEYNOTE SPEAKERS** Chair: Hank Bass Pages 27 & 28.
10:30 AM	Introduction
10:40 AM	**Anna Stepanova, North Carolina State University** [KS4] *Leveraging synthetic biology to monitor and control plant hormone activity*
11:40 AM	**Natalia de Leon, University of Wisconsin-Madison** [KS5] *Plant breeding and the infinitesimal model: cause or consequence*
12:30 PM – 1:30 PM	**LUNCH** (Ballrooms B&C) MaGNET Lunch with Keynote Speakers (301A) MGMSC Meeting (301B) Maize Genetics Mentoring Program Networking Lunch (303)

Saturday, March 2, 2024 (continued)

1:30 PM – 4:30 PM **POSTER SESSION 2** (Ballrooms B&C)

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 Ballrooms B&C

4:30 PM – 6:00 PM **COMMUNITY SESSION - Maize Genetics Cooperative**
Marna Yandeau Nelson, MGC BoD Chair (Ballroom A)

6:00 PM – 7:00 PM **DINNER** (Ballrooms B&C)
Corteva Student/Postdoc Dinner (301A)
Syngenta Student/Postdoc Dinner (301B)

7:00 PM – 8:20 PM **SESSION 8 – MAIZE EVOLUTION**
Chair: Matthew Hufford Talks 16-19. Pages 45-48.

7:00 PM **Robert Martienssen, Cold Spring Harbor Laboratory** [T16]
Teosinte pollen drive guides maize diversification and domestication by RNAi

7:20 PM **Ruth Epstein, Cornell University** [T17]
How recombination facilitated the domestication of maize.

7:40 PM **Michael Bushe, University of Wisconsin-Madison** [T18]
Maize development is controlled by an ancient nutrient signaling pathway

8:00 PM **Samantha Snodgrass, Iowa State University** [T19]
Fractionation in the Tripsacinae subtribe

8:20 PM – 8:40 PM **BREAK** Ballroom ABC Lobby

8:40 PM – 9:40 PM **SESSION 9 – ROOTS AND FRIENDS**
Chair: Stephanie Klein Talks 20-21. Pages 49-50.

8:40 PM **Michelle Cho, Washington University Saint Louis** [T20]
ZmIRA1, a new root system architecture gene identified by phenomics

9:00 PM **Jason Wallace, University of Georgia** [T21]
Genetic and environmental impacts on the maize microbiome across the United States

9:20 PM **Poster Lightning Talks**

9:40 PM – 12:00 AM **INFORMAL POSTER VIEWING & HOSPITALITY** (Ballrooms B&C)
10:00 PM – 12:00 AM **TRIVIA!!** (Ballrooms B&C)

Posters should be taken down by 12 AM!

Sunday, March 3, 2024

7:00 AM – 8:20 AM	**BREAKFAST**	(Ballroom ABC Lobby)

8:00 AM – 9:40 AM	**SESSION 10 – THE REGULATED GENOME** Chair: Sarah Jensen	Talks 22-26. Pages 51-55.
8:00 AM	**Andrea Gallavoti, Rutgers University** *Exploring the cis-regulatory landscape of the B73 and Mo17 genomes*	[T22]
8:20 AM	**Sohyun Bang, University of Georgia** *Understanding the role of ZmWUS1 and cis-regulatory elements in maize inflorescence development at single-cell resolution*	[T23]
8:40 AM	**Sunil Kenchanmane Raju, New York University** *Integrating single-cell transcriptomes links organ-specific gene expression with function*	[T24]
9:00 AM	**Gwonjin Lee, University of Florida** *Meiotic recombination differs between sexes in maize.*	[T25]
9:20 AM	**Oliver Marchus, Ohio State University** *x1 encodes a putative RNA-binding protein required for paramutations at multiple loci*	[T26]
9:40 AM – 10:00 AM	**BREAK**	Ballroom ABC Lobby

10:00 AM – 11:40 AM	**SESSION 11 – METABOLIC REGULATION** Chair: Sherry Flint-Garcia	Talks 27-30. Pages 56-59.
10:00 AM	**Elsbeth Walker, University of Massachusetts, Amherst** *Strigolactone control of iron homeostasis in maize.*	[T27]
10:20 AM	**Ankita Abnave, University of Toledo** *Upper level and cross hierarchical regulation of predominantly expressed phenolic genes in maize*	[T28]
10:40 AM	**Manwinder Singh Brar, Clemson University** *Elucidating the metabolomic and single-cell transcriptomic landscape of maize leaf senescence*	[T29]
11:00 AM	**Jonathan Gent, University of Georgia** *Potent pollen gene regulation by DNA glycosylases in maize*	[T30]
11:20 AM	**CLOSING REMARKS (Rubén Rellán Álvarez and Sherry Flint-Garcia)**	
11:30 AM	**ADJOURNMENT**	

Poster List

Biochemical and Molecular Genetics

P1	**Jacob Olson** <olson169@purdue.edu>	*Annotation of gene-metabolite functional relationships using multi-omics mapping of the Sorghum Bioenergy Association Panel*
P2	**Jazmin Abraham-Juarez** <jazmin.abraham@cinvestav.mx>	*Autoimmune mutants reveal new immunity pathways in maize*
P3	**Juliana Yassitepe** <juliana.yassitepe@embrapa.br>	*Challenges and insights from establishing a maize transformation laboratory*
P4	**Nadia Mourad Silva** <nmourad@ufl.edu>	*Changes in sorbitol metabolism impact kernel sink strength and seed size*
P5	**Allison Barnes** <allison.barnes@usda.gov>	*Characterization of the maize membrane lipidome in a large collection of introgression lines derived from crosses between B73 and traditional maize varieties and teosintes*
P6	**Irina Makarevitch** <imakarevitch01@hamline.edu>	*Characterizing expression of meristem genes in a Lig5 maize mutant*
P7	**Hui Liu** <huil@ksu.edu>	*Comparative study of approaches for gene activation in maize*
P8	**Shivreet Kaur** <skaur7@ncsu.edu>	*Delving into the biochemical underpinnings of disease resistance for southern leaf blight in maize*
P9	**Charles Hunter** <cthunter3@gmail.com>	*Disruption of allene oxide cyclase results in jasmonic acid deficiency despite incomplete loss of 12-OPDA*
P10	**Elad Oren** <eo235@cornell.edu>	*Elucidating freezing tolerance in tripsacum: Insights from combined transcriptome and proteome*
P11	**Anson Siu** <acsiu@wisc.edu>	*Empirical assessment of the Wisconsin Crop Innovation Center high throughput maize gene editing pipeline*
P12	**Natalie Deans** <natalie.deans@uga.edu>	*Engineering a terpene-depleted chassis in tomato fruit for production of high value terpenes*
P13	**Jonathan Ojeda-Rivera** <joo29@cornell.edu>	*Engineering root N remobilization after grain filling to decrease greenhouse gas emissions and N leaching in corn agriculture*
P14	**Yuguo Xiao** <yxiao@danforthcenter.org>	*Exploring the genetics of drought resilience in sorghum*
P15	**Gerardo Gonzalez** <ggg7@hawaii.edu>	*Finding a high throughput DNA extraction method for maize*
P16	**Lincoln Koehler** <lck4@illinois.edu>	*Functional genomics of coordinated nitrogen and phosphorous signaling in maize*
P17	**John Gray** <john.gray5@utoledo.edu>	*GRASSIUS 2.0 Knowledgebase: Updated resources and tools for regulomics in the grasses*
P18	**Rebecca Selby** <becca.selby@syngenta.com>	*Gene editing of two maize chalcone synthase paralogs results in conditional male sterility*
P19	**Amanpreet Kaur** <kaur60@purdue.edu>	*Gene expression identifies a role of maize ortholog of dwarf and low tillering (gras42) in brassinosteroid signaling*
P20	**Bharath Kunduru** <bkundur@clemson.edu>	*Genetic architecture of stalk lodging resistance in maize*
P21	**Jason Punskovsky** <jpunskovsky576@gmail.com>	*Genetic interaction analysis of maize meristem regulators*
P22	**Dirk Winkelman** <dwink@iastate.edu>	*Glossy2 and Glossy2-like demonstrate both unique and overlapping functions in maize cuticular wax formation.*

P23	**Lorenzo Concia** <lconcia@tacc.utexas.edu>	*High-sensitivity transcriptional profiling of maize root tips via nascent and nuclear RNA sequencing*
P24	**Lina Gomez-Cano** <gomezca5@msu.edu>	*Identification of genes associated with phenolic metabolism*
P25	**Brian Dilkes** <bdilkes@purdue.edu>	*Identifying physiologically relevant transcriptional regulatory mechanisms in tetrapyrrole biosynthesis using natural and induced variation in Mg2+ chelatase in maize.*
P26	**Ella Hampson** <ellagh@hawaii.edu>	*Identifying the functional determinants of ZmEhd1 - a putative maize flowering time activator using a protoplast system.*
P27	**Jaclyn Nicole Uy** <uyjnr69@hawaii.edu>	*Improving transformation and regeneration of tropical inbred lines using calcium channel blockers*
P28	**Sylvia Morais de Sousa Tinoco** <sylvia.sousa@embrapa.br>	*Inoculation effect of phosphate-solubilizing bacteria on the microbiota of maize cultivated under different phosphate fertilization conditions*
P29	**Sarah Lipps** <slipps@illinois.edu>	*Insights into genomic regions associated with resistance and susceptibility to Gibberella ear rot*
P30	**Huda Ansaf** <hah34@umsystem.edu>	*Integration of proteomics and phosphoproteomics provides insight into the protein-bound amino acid regulation in the opaque2 mutant*
P31	**Fausto Rodriguez-Zapata** <frodrig4@ncsu.edu>	*Introgression of a highland maize chromosomal inversion decreases flowering time in a phosphorus-independent manner, and leads to minor perturbations of the leaf phosphorus starvation response transcriptome.*
P32	**Ruthie Stokes** <rstokes@ncsu.edu>	*Investigating the expression regulation and enzymatic activity of the adaptive gene hpc1*
P33	**Diana Ramirez Segovia** <daramir3@ncsu.edu>	*Investigating the maize systemic disease response to the southern leaf blight pathogen, Cochliobolus heterostrophus.*
P34	**Ankush Sangra** <ankush.sangra@uga.edu>	*Investigating the role of the cis-regulatory modules and hopscotch in maize domestication QTL teosinte branched1*
P35	**Estefania Elorriaga** <eelorri@ncsu.edu>	*Investigation of tissue-specific translation regulation by uORFs in maize*
P36	**Zachary Turpin** <zachturpin92@gmail.com>	*MOA-seq identifies candidate cis-elements associated with response to submergence.*
P37	**David Skibbe** <david.skibbe@syngenta.com>	*Maize cytoplasm genotype is a key determinant of transformability*
P38	**Anna Block** <anna.block@usda.gov>	*Maize Terpene Synthase 1 impacts insect pest behavior via the production of monoterpene volatiles β-myrcene and linalool.*
P39	**Israel Arellano** <i_a67@tamu.edu>	*Maize-specific LOX4 and LOX5 genes subfunctionalize to play contrasting roles in defense against necrotrophic and hemibiotrophic pathogens*
P40	**Tianxiao Yang** <tianxiao.yang@ufl.edu>	*Maize rough endosperm6 (rgh6) encodes a predicted DEAD-box RNA helicase and affects miRNA processing in endosperm development*
P41	**Meghan Brady** <meghan.brady@uga.edu>	*Minus directed kinesin allows chromosome 10 haplotypes to compete*
P42	**Marie Javelle** <marie.javelle@limagrain.com>	*Modular base editing*
P43	**Rajdeep Khangura** <rkhangur@purdue.edu>	*Molecular identification of two Mu-suppressible alleles of lesion28 as insertions in UROPORPHYRINOGEN III SYNTHASE*

P44	**Jeonghwa Oh** <jeonghwa.oh@cnrs.fr>	*Natural variation in maize root hydraulic architecture may offer new insights into plant drought responses*
P45	**Tyler Ferris** <tyler.ferris@huskers.unl.edu>	*Nutritional and biophysical characteristics of CRISPR-generated low kafirin, waxy sorghum*
P46	**Juliana Yassitepe** <juliana.yassitepe@embrapa.br>	*Phenotyping transgenic events for drought resistance*
P47	**Rohit Kumar** <mohank@clemson.edu>	*Proteomic and metabolomic landscape of the arbuscular mycorrhizal symbiosis in maize roots*
P48	**Namrata Jaiswal** <namrata.jaiswal@usda.gov>	*Pseudomonas syringae pv. tomato DC3000 induces defense responses in diverse maize inbred lines*
P49	**Brianna Griffin** <bdg@iastate.edu>	*Roles of REL2 mediated transcriptional co-repression in maize immunity*
P50	**Katherine Gray** <kgray3@ufl.edu>	*Roles of vacuolar invertase genes (Ivr1 & Ivr2) in the pollination biology of maize*
P51	**Mingsheng Peng** <mingsheng.peng@bayer.com>	*Site-directed integration of microbial HemG-type protoporphyrinogen IX oxidase (PPO) at a target site in corn genome creates tolerance to PPO-inhibiting herbicides*
P52	**Taylor Scroggs** <taylor.scroggs@uga.edu>	*Systematic exploration of transcription factor function in maize*
P53	**Raksha Singh** <raksha.singh@usda.gov>	*Tar spot and fisheye lesions exhibit distinct yet overlapping transcriptomic signatures, suggesting a complex interplay of shared and specific pathways modulated by Phyllachora maydis.*
P54	**Olga Zimina** <oz32@cornell.edu>	*Targeting meiotic crossovers in maize*
P55	**Wen-Yu Liu** <wliu34@ncsu.edu>	*The maize E3 ligase ZmCER9 specifically targets activated NLRs for degradation*
P56	**Holly Anderson** <hollyaa2@illinois.edu>	*Transcriptomic variation during kernel development in near-isogenic purple maize cultivars*
P57	**James Birchler** <birchlerj@missouri.edu>	*Transformation of Fast Flowering Mini Maize (FFMM)*
P58	**Mateusz Zelkowski** <mz548@cornell.edu>	*Unlocking meiotic recombination in the maize genome.*

Cell and Developmental Biology

P59	**George Chuck** <georgechuck@berkeley.edu>	*A KNOX-BLH homeodomain complex targets a major domestication locus*
P60	**Xiaosa Xu** <xjkxu@ucdavis.edu>	*A nuclear moonlighting function of maize trehalose-6-phosphate phosphatase in inflorescence branching*
P61	**Zongliang Chen** <zlchen@waksman.rutgers.edu>	*A regulatory module of LITTLE ZIPPER and ROLLED LEAF1/REVOLUTA controls ligule development in maize*
P62	**yuebin wang** <1310214636@qq.com>	*A spatial transcriptome map of the developing maize ear*
P63	**Ryaniah Ellis** <ryaniah1.ellis@famu.edu>	*Analysis of Pistachio root proteome to salt stress*
P64	**Harrison Bell** <bellhar@oregonstate.edu>	*Automated image analysis of maize pollen for phenotypic measurements*
P65	**Thu Tran** <tran@cshl.edu>	*Catalytic and non-catalytic TREHALOSE-6-PHOSPHATE SYNTHASES (ZmTPSs) interact with RAMOSA3 to control embryo and post-embryo development*

P66	**Alec Chin-Quee** <chinquee2@ufl.edu>	*Cause and consequences of abnormal Meiosis II division in bige7 heterozygotes*
P67	**Sinead Cahill** <sbcahill@ucsd.edu>	*Characterizing the phenotypic rescue of D-erythrose in salt-stressed maize and its role in stress-response regulation and development*
P68	**Pasquale Hendrawinata** <hendrawp@oregonstate.edu>	*Cloning the classic maize metaxylem mutant, wilted1 (wi1) by chromosome walking and RNA sequencing*
P69	**Emily Wheeler** <eamarkha@ncsu.edu>	*Comparison of DNA replication timing profiles between B73 and NC350 maize lines*
P70	**John Hodge** <jghodge@illinois.edu>	*Coupling computer vision with spatial analysis of cell patterning allows the genetic basis for stomatal density to be dissected into component traits related to known developmental mechanisms in maize*
P71	**Clarice Gonzales** <clariceg@iastate.edu>	*Crosstalk between auxin and jasmonic acid in maize root development*
P72	**Lily O'Connor** <loconnor@danforthcenter.org>	*Defining the gene regulatory networks of bHLH122 and bHLH51 in maize anther development*
P73	**Irene Ikiriko** <iikiriko@udel.edu>	*Determining the impact of plant architecture on lifespan stalk flexural stiffness*
P74	**Taran Kermani** <kermani@udel.edu>	*Development of a lineage tracing line for maize brace root developmental studies*
P75	**Jared Carter** <Jared.Carter@Syngenta.com>	*Development of an accessible and scalable maize pollen storage technology*
P76	**Kyle Swentowsky** <swentow@cshl.edu>	*Developmental genetics and genomics of perennial regrowth in Zea diploperennis*
P77	**Lukas Evans** <Le95@cornell.edu>	*Drawing the line: interactions of maize transcription factors to coordinate proximodistal and mediolateral patterning in the maize leaf*
P78	**Nandhakumar Shanmugaraj** <shanmug@cshl.edu>	*Elucidating the role of TERMINAL EAR1 (TE1) in maize development and stress*
P79	**Devin O'Connor** <doconnor@pairwise.com>	*Engineering dominant maize yield-component improvement with gene editing*
P80	**Jessica Ji** <jjessica@iastate.edu>	*Fast Turnaround Maize Transformation Service by Crop Bioengineering Laboratory at Iowa State University*
P81	**Daniela Meson De La Fuente** <dmeson@oakland.edu>	*Functional and genetic analysis of alternatively spliced transcript isoforms of a conserved RNA Binding Motif Protein 48 (RBM48) essential for the splicing of U12-type introns*
P82	**Emilia Pierce** <emiliap@udel.edu>	*Functional characterization of maize nitrogen transporters*
P83	**Yan Zhou** <yzhou86@iastate.edu>	*Genetic regulation of self-organizing azimuthal canopy orientations and their impacts on light interception in maize*
P84	**Jaspreet Sandhu** <jsandhu@danforthcenter.org>	*Growth hormones BR and GA modulate spikelet meristem identity through the RAMOSA1 pathway in Setaria and maize*
P85	**Kevin Begcy** <kbegcy.padilla@ufl.edu>	*Heat stress at the bicellular stage inhibits sperm cell development and their transport into pollen tubes*
P86	**Thomas Dresselhaus** <thomas.dresselhaus@ur.de>	*Heat stress induced pollen tube growth arrest and sterility supported by a MaizeStressDB*
P87	**Fisher Stines** <fisher.stines@syngenta.com>	*Herbicide safener Metcamifen significantly improves the recovery of doubled haploid maize seedlings via in vitro culture*

P88	**Ella Townsend** <ellatownsend90@gmail.com>	*High-throughput root phenotyping to investigate phenotypic relationships between early auxin response and adult crown root phenotyping*
P89	**Brian Zebosi** <bzebosi@iastate.edu>	*Individual and collective tissue-specific roles of BRASSINOSTEROID INSENSITIVE1 (BRI1) receptor-like kinase to plant development and BR signaling in maize.*
P90	**Charles Maus** <mausc17@students.ecu.edu>	*Investigating links between pectin dynamics and meristem activity in maize*
P91	**Andrea Sama** <asama@ucsd.edu>	*Investigating the effect of environmental stress on metabolite localization and signaling in plants*
P92	**Richie Eve Ragas** <rgr86@cornell.edu>	*Investigating the role of higher-WOX3 transcription factors during robust patterning of leaf-margin orientation and tassel spikelet development*
P93	**Lizeth Dominguez** <lizethd2@illinois.edu>	*Is more always better? Progress and promise of autotetraploid sweet sorghum*
P94	**Vital Nyabashi** <vitalnya@iastate.edu>	*K-means clustering analysis of gene expression across endosperm development*
P95	**Stephanie Martinez** <smart046@ucr.edu>	*KATANIN's role in cell division positioning and cell elongation in maize*
P96	**Xiaosa Xu** <xjkxu@ucdavis.edu>	*Large-scale single-cell profiling of stem cells uncovers regulators of plant shoot development*
P97	**Thanduanlung Kamei** <thanduan@udel.edu>	*Linking brace root development to function*
P98	**Josh Strable** <jjstrabl@ncsu.edu>	*Maize ETHYLENE INSENSITIVE3-LIKE genes are central regulators of shoot growth*
P99	**Lander Geadelmann** <landerg@iastate.edu>	*Maize developmental transcription factors affect and interact with aberrant phyllotaxy1 in regulating phyllotaxy*
P100	**Camila Medina** <medinamm@oregonstate.edu>	*Making good connections: How transverse veins span the maize leaf vascular network*
P101	**Arif Ashraf** <arif.ashraf.opu@gmail.com>	*Nuclear membrane proteins and their functions beyond the nuclear membrane*
P102	**Jingjing Tong** <jjtong@udel.edu>	*PEG-mediated transient gene expression in maize root protoplast*
P103	**Siddique Aboobucker** <siddique@iastate.edu>	*Parallel spindle genes restore haploid male fertility – removing a bottleneck in doubled haploid technology*
P104	**Ramesh Katam** <ramesh.katam@famu.edu>	*Phytoextraction potential of Quinoa and wheat grown under saline conditions as a measure for adaptation to salt stress*
P105	**Theresa Clark** <tclark@cshl.edu>	*Plant regeneration using morphogenic regulators*
P106	**Penelope Lindsay** <lindsay@cshl.edu>	*Regulation of maize ear development by a CLAVATA-related receptor complex*
P107	**Jason Gregory** <jasongregoryj@gmail.com>	*Regulation of meristem size by the REL2corepressor family*
P108	**Ruqiang Zhang** <rz444@cornell.edu>	*Single-cell RNAseq analysis and dynamic modeling of leaf-angle variation in the maize leaf canopy*
P109	**Wesley Neher** <wnehe001@ucr.edu>	*The maize preligule band is subdivided into distinct domains with contrasting cellular properties prior to ligule outgrowth*
P110	**Shawn Kaeppler** <smkaeppl@wisc.edu>	*The Wisconsin Crop Innovation Center: a public resource for maize transformation and gene editing research*

P111	**Liuyang Chu** <lchu@uni-bonn.de>	*The boron deficiency response during primary root development of maize*
P112	**Hailong Yang** <hailongyang@umass.edu>	*The cis-regulatory evolution of GRASSY TILLERS1 (GT1) over deep time*
P113	**Fernanda Ghenov** <fghenov@hawaii.edu>	*The flowering phenotypes of temperate and tropical maize grown in short and long day field environments*
P114	**J.Aaron Avalos-Calleros** <aaronac089@ksu.edu>	*The genetic basis of callus development and resources for genetic transformation of maize*
P115	**Harrison Flieg** <fliegh@oregonstate.edu>	*The heterofertilization and kernel abortion phenotypes in maize gex2 mutants are consistent with differential fertilization recovery of egg and central cells.*
P116	**Heather Jones** <s1808249@ed.ac.uk>	*The maize fusedleaf1 mutant*
P117	**Emma Klaas** <eklaas43@gmail.com>	*The role of the plant growth hormones auxin and cytokinin in maize mutants lsn1 and Hsf1*
P118	**Emily Wear** <emily_wear@ncsu.edu>	*Tracking S-phase progression: Sequential dual labeling of replicated DNA with EdU and BrdU in maize root tips*
P119	**Sessen Daniel Iohannes** <iohannes@cshl.edu>	*Tradeoffs, balance and drift: the genetics of paralogous compensation in the maize meristem*
P120	**Nicholas Gladman** <gladman@cshl.edu>	*Transcriptomic profiles of developing meristems across sorghum accessions reveal nuanced regulatory pathways towards panicle morphology*
P121	**Denise Caldwell** <denisecaldwell74@gmail.com>	*Uncovering the infection strategy of Phyllachora maydis during maize colonization: A comprehensive analysis*
P122	**Diana Ruggiero** <ruggiedi@oregonstate.edu>	*Understanding leaf vascular density through quantitative genetics and high-throughput phenotyping*
P123	**Trisha McAllister** <trishamcallister13@gmail.com>	*Unravelling the role of LIGULELESS2 in maize*
P124	**David Zimmerman** <djz3@illinois.edu>	*Vasculature connection in maize "Twin-Grafts"*
P125	**Nicholas Francis** <francini@oregonstate.edu>	*WHETSTONE1 (WTS1), a single dominant locus from the W22 background suppresses Wavy auricle in blade2 (Wab2)*
P126	**Leslie Harris** <harriles@oregonstate.edu>	*Wavy Auricle in Blade2 (Wab2) overexpresses tcptf15 and is suppressed by one copy of wavy auricle in blade1 (wab1) loss-of-function*
P127	**Norman Best** <norman.best@usda.gov>	*ZmCAND1 is hyposensitive to auxin treatment and controls maize growth and development*
P128	**Craig Cowling** <ccowling@iastate.edu>	*ZmPILS6 is an auxin efflux carrier required for maize morphogenesis*
P129	**Prameela Awale** <pa96f@umsystem.edu>	*tasseless5 (tls5) affects plant height and reproductive development in maize by regulating internode elongation and tassel emergence.*

Computational and Large-Scale Biology

P130	**Carson Andorf** <carson.andorf@usda.gov>	*MaizeGDB: AI-driven resources for maize*
P131	**Carson Andorf** <carson.andorf@usda.gov>	*A unified VCF dataset from 1,500 diverse maize accessions and resources to explore the genomic landscape of maize*
P132	**Jack Gardiner** <jack.m.gardiner@gmail.com>	*MaizeMine: Tools for exploring genomic datasets from the maize research community*

P133	**Olivia C. Haley** <olivia.haley@usda.gov>	*The prediction of Zea mays (maize) and Fusarium graminearum host-pathogen protein-protein interactions using fine-tuned protein language models and diffusion*
P134	**Rita Hayford** <rita.hayford@usda.gov>	*Identification and annotation of stress response transcriptional regulatory mechanisms in maize*
P135	**Ethalinda Cannon** <Ethy.Cannon@usda.gov>	*Pan-gene and gene family data at MaizeGDB*
P136	**Alexandria Tran** <tran30@illinois.edu>	*A developmental series transcriptomic study of nitrogen-related heterosis*
P137	**Linqian Han** <linqian.han@wsu.edu>	*A multi-omics integrative network map of maize*
P138	**Niaz Chowdhury** <nchowdhury2@huskers.unl.edu>	*A multi-organ maize metabolic model connects temperature stress with energy production and reducing power generation*
P139	**Jodi Callwood** <jodicall@iastate.edu>	*Advancing maize phenotyping through automation and data analysis*
P140	**Keting Chen** <kchen@iastate.edu>	*Are the non-coding regions really non-coding? Large-scale identification of orphan genes from B73 v5 genome*
P141	**Xianran Li** <xianran.li@usda.gov>	*BRIDGEcereal webapp for survey and graph indel-based haplotypes from pan-genomes*
P142	**Travis Wrightsman** <tw493@cornell.edu>	*Benchmarking across-species RNA expression prediction within maize and its wild relatives*
P143	**Terry Casstevens** <tmc46@cornell.edu>	*Breeders' genomic hub: Integrating analysis tools with data*
P144	**Joseph Cristiano** <jcristia@udel.edu>	*Brobot & RootTaggingGui: An image acquisition and processing pipeline for high-volume phenotyping*
P145	**Hank Bass** <bass@bio.fsu.edu>	*Chromatin structure and particle mapping in maize root tip nuclei using MNase digestion assays.*
P146	**Arun Seetharam** <arnstrm@iastate.edu>	*Chromosome-level assembly and comprehensive annotation of the W22 and PH207 maize inbreds*
P147	**Leigh Mickelson-Young** <lamickel@ncsu.edu>	*Comparison of rRNA depletion methods in maize*
P148	**Arnaud Desbiez-Piat** <arnaud.desbiez-piat@inrae.fr>	*Coupling ecophysiology and genetics in a digital environment for modeling crop adaptation*
P149	**John Fowler** <fowlerjo@oregonstate.edu>	*EarVision.v2 detects spatial effects of pollen mutations on kernel distribution across the maize ear*
P150	**Zong-Yan Liu** <zl843@cornell.edu>	*Enhancing plant genome annotation through DNA language model: A focus on arabidopsis and maize*
P151	**Daniel Kick** <daniel.kick@usda.gov>	*Ensembles of deep learning, machine learning, and linear models outperform individual models for maize yield prediction in diverse environments*
P152	**Justin Walley** <jwalley@iastate.edu>	*Genetic determinants of Northern Corn Leaf Blight resistance revealed by multi-scale systems genetics*
P153	**Joan Barreto Ortiz** <jbarreto@umn.edu>	*Genomic analysis of stability in grain composition in maize*
P154	**Doreen Ware** <ware@cshl.edu>	*Gramene PanMaize: One-stop pan-genome browser for exploring the rich genetic diversity in maize*
P155	**Katie Murphy** <kmurphy@danforthcenter.org>	*High-throughput phenotyping of maize stress responses in controlled environments*
P156	**Lara Brindisi** <ljbrindisi@gmail.com>	*Identification of cold-adapted proteins in Poaceae using machine learning and comparative genomics*

P157	**Rommel Jr. Garrido** <garri318@umn.edu>	*Impacts of structural variants on accurate SNP imputation*
P158	**Sarah Jane McMorrow** <smm477@cornell.edu>	*Improving polyploid and heterozygous genome interpretation using hidden Markov models*
P159	**Sanzhen Liu** <liu3zhen@ksu.edu>	*K-mer trait association mapping and prioritization of candidate genes with Homotools for structural comparison*
P160	**Rajdeep Khangura** <rkhangur@purdue.edu>	*Life, the universe, and everything for $42: WideSeq mapping of maize mutants*
P161	**Zhikai Liang** <zhikai.liang@ndsu.edu>	*Mapping genomic elements in response to heat stress in maize*
P162	**Nikee Shrestha** <nshrestha5@huskers.unl.edu>	*Mapping loci controlling sorghum seed color using computer vision models pre-trained in distantly related grain crops*
P163	**Jeff Bennetzen** <maize@uga.edu>	*Microbial Partner (MiPner) analysis for the discovery and study of microbe-microbe interactions*
P164	**J. Vladimir Torres-Rodriguez** <vladimir.torres@unl.edu>	*Population level gene expression can repeatedly link genes to functions in maize*
P165	**Michael Tross** <mtross2@huskers.unl.edu>	*Predicting cell type specific expression of maize gene models using DNA sequence*
P166	**Jingjing Zhai** <jz963@cornell.edu>	*Prioritizing deleterious mutations in Maize by DNA language model*
P167	**Brandon Webster** <webst250@msu.edu>	*Remote sensing for response to nitrogen fertilizer in maize*
P168	**Doreen Ware** <ware@cshl.edu>	*Swift pan-genomic methods for comprehensive genome annotation in maize genomes*
P169	**Josue Fernandez Canela** <jf68753@uga.edu>	*The establishment and progression of microbial communities associated with the developing seedling roots of maize and sorghum*
P170	**Noelle Svoboda** <nsvoboda6@gmail.com>	*The maize rhizoplane microbial community*
P171	**Kate Eastman** <eastman@purdue.edu>	*Transcriptomic responses in maize and green foxtail to Poaceae-adapted and generalist lepidopteran herbivores*
P172	**Quinn Johnson** <qyj2@cornell.edu>	*Unraveling the chromatin environment of double-strand breaks leading to crossovers and non-crossovers*
P173	**Grace Vincent** <gmvincen@ncsu.edu>	*Utilizing hyperspectral field imagery for accurate southern leaf blight severity grading in maize*

Cytogenetics

P174	**Juli Jing** <jj696@cornell.edu>	*An F-box protein ACOZ1 functions in crossover formation by ensuring proper chromosome compaction during maize meiosis*
P175	**James Birchler** <birchlerj@missouri.edu>	*B chromosome mediated effects on A chromosomes*
P176	**Hafiza Sara Akram** <ha20be@fsu.edu>	*Elucidating the relationship between DNA replication timing within S-phase and the spatial organization of chromatin within maize nuclei*
P177	**Hua Yang** <yanghu@missouri.edu>	*Global modulation of gene expression in aneuploidy combinations in maize*
P178	**Yibing Zeng** <yz77862@uga.edu>	*Stability of chromosome segregation by synthetic maize centromeres*
P179	**James Birchler** <birchlerj@missouri.edu>	*The long journey of Barbara McClintock's slides defining the NOR*

P180 **Hua Yang** <yanghu@missouri.edu> — *Unveiling the molecular mechanism of the B chromosome nondisjunction—a component of its drive mechanism*

Education & Outreach

P181 **Aimee Schulz** <ajs692@cornell.edu> — *A seed dispersal game: curriculum for teaching plant domestication and adaptation to students of all ages*

P182 **Daniel Levin** <dlevin2@umd.edu> — *Broader impact through teacher education: Teachers confront the messiness of scientific practice*

P183 **Edward Buckler** <esb33@cornell.edu> — *CENSA: Create a sustainable food system for nitrogen: Moving synthetic nitrogen from the field to barn*

P184 **Vivian Bernau** <vivian.bernau@usda.gov> — *Characterizing and distributing maize diversity: The NCRPIS maize collection*

P185 **Katherine Gray** <kgray3@ufl.edu> — *Evolution of genome editing regulations: Comparative analysis in model countries*

P186 **Marlies Wouters** <mwouters@inari.com> — *From the Inari gene editing toolbox: CRISPR/Cas-mediated enhancer insertion in maize*

P187 **Mark Lubkowitz** <Mlubkowitz@smcvt.edu> — *Leaf angle and curricular innovation: a CURE for research and undergraduate education*

P188 **Brandi Sigmon** <bsigmon2@unl.edu> — *MARVEL: An inclusive student persistence framework to close equity gaps in STEM workforce development*

P189 **Bianca Sheridan** <bsheridan@fsu.edu> — *The Maize-10-Maze project, an educational public chromosome map garden with mutants of maize at FSU and online.*

Quantitative Genetics & Breeding

P190 **Sergio Perez-Limoon** <sjp6181@psu.edu> — *A MAGIC Population to study functional diversity in mexican native maize*

P191 **Qi Mu** <qimu@udel.edu> — *A MYB transcription factor underlying plant height in sorghum and maize reveals functional connections across natural alleles, mutant, and edited alleles*

P192 **Alexander Liu** <aliu@danforthcenter.org> — *A Rootless1 knockdown allele affects maize nodal root development, changing root system architecture and function.*

P193 **Nate Korth** <nate.korth@gmail.com> — *A case for GERM: calculating Genotype by Environment by Rhizosphere Microbiome interactions to improve crop yield and resilience.*

P194 **Grace Nystrom** <gnystrom@wisc.edu> — *A cat-and-mouse game for the stay-green phenotype in maize hybrids*

P195 **Lauren Whitt** <lwhitt@danforthcenter.org> — *A comparative approach for selecting orthologous candidate genes underlying signal in elemental accumulation genome-wide association studies across multiple species*

P196 **Zachary Traylor** <zbtyxb@umsystem.edu> — *A new ethanol extraction method in maize using lab-scale, dry-grind technology*

P197 **Sarah Oliver** <sloxmd@umsystem.edu> — *A tale of two functions: Exploring Asparagine's impact on human health and the environment*

P198 **Yawei Li** <yaweil@iastate.edu> — *AI-driven image-based high-throughput phenotyping and genetic analysis of plant architecture in maize*

P199 **Eduardo Oliveira** <eduardovo@usp.br> — *Analysis of B73 seed phenotype changes in response to tropical conditions*

P200	**August Thies** <athies@danforthcenter.org>	*Analyzing the impact of 40 years of breeding and low nitrogen environments on maize root system architecture using X-ray imaging*
P201	**Hannah Pil** <hdpil@ncsu.edu>	*BZea: A diverse teosinte introgression population for investigating agronomic traits in maize*
P202	**Paul Scott** <paul.scott@usda.gov>	*Breeding corn for organic production systems*
P203	**Jonathan Niyorukundo** <niyorukundoj@gmail.com>	*Breeding for color and improved protein content in sweet corn and popcorn*
P204	**Jagdeep Singh Sidhu** <jagdeeproots@gmail.com>	*Breeding for root traits in cereal crops*
P205	**Cinta Romay** <mcr72@cornell.edu>	*CERCA - Circular Economy that Reimagines Corn Agriculture*
P206	**Cristian Zavala Espinosa** <c.zavala@cgiar.org>	*CIMMYT Maize Lines (CMLs): A fundamental resource for maize breeders and geneticists*
P207	**Melissa Draves** <madcfr@umsystem.edu>	*Characterizing heirloom maize of the United States*
P208	**Amparo Daza** <dazacastanon@wisc.edu>	*Cold tolerance evaluation in maize through seedling establishment strategies*
P209	**Mariana Vianna** <viannam@ufl.edu>	*Comparing k-mer-based and SNP-based GWAS approaches to identify structural variants in sweet corn*
P210	**Kiara Kappelmann** <kiarak@iastate.edu>	*Comprehensive kernel analysis in large DH populations derived from intercrossing germplasm enhancement of maize lines*
P211	**Jinliang Yang** <jinliang.yang@unl.edu>	*Contribution of tropical alleles to heterosis in root traits and root-associated microbial traits under varied nitrogen conditions*
P212	**Akanksha Singh** <singh412@purdue.edu>	*D16 – A game-changer in the quest to generate short-stature corn hybrids*
P213	**Xia Zhang** <xia.zhang@syngenta.com>	*Developing transformable haploid inducers to meet commercial-scale genome editing in maize*
P214	**Nirwan Tandukar** <ntanduk@ncsu.edu>	*Discovering phosphorus efficiency alleles in maize and sorghum by combining multiple omics datasets and advanced modeling statistics*
P215	**Patrick Sydow** <pws5408@psu.edu>	*Dissecting the genetic architecture of root traits in the MEXIMAGIC population*
P216	**Irene Gentzel** <gentzel.3@osu.edu>	*Effects of maize chlorotic mottle virus and potyvirus resistance on maize lethal necrosis disease*
P217	**Greg Schoenbaum** <gregorys@iastate.edu>	*Effects of plot orientation and leaf angle on maize grain yield*
P218	**Solomon Oloruntoba Samuel** <sosamuel@ncsu.edu>	*Examining the dynamics of microbe-driven heterosis in maize*
P219	**Rafaela Prado Graciano** <rafaela.graciano@ufl.edu>	*Exploring the application of phenomic selection in corn breeding.*
P220	**Melaku Gedil** <m.gedil@cgiar.org>	*Expression analysis of key genes controlling resistance to Striga hermonthica in tropical maize inbred lines*
P221	**Silvio Salvi** <silvio.salvi@unibo.it>	*Fine mapping and positional cloning of a major root system architecture QTL in maize*
P222	**Kirsten Hein** <kirsten.hein@colostate.edu>	*Functional genetics of G×E QTL for root system architecture in maize*
P223	**Tao Zhong** <tzhong3@ncsu.edu>	*GWAS analysis reveals resistant genes to maize southern leaf blight*

P224	**Daniel Laspisa** <Daniel.Laspisa@uga.edu>	*Genetic determinants of aerial root formation in maize: Prospects for harnessing biological nitrogen fixation*
P225	**Leticia Prada de Miranda** <leticiapradam@gmail.com>	*Genetic potential of tropical, temperate, and mixed maize populations evaluated in tropical environments*
P226	**Dayane Cristina Lima** <dclima@wisc.edu>	*Genetic analysis of yield, combining ability, and agronomic performance in non-Stiff Stalk elite germplasm in a factorial mapping population*
P227	**Hongyu Jin** <hjin5@huskers.unl.edu>	*Genetic elucidation of maize leaf length and width traits*
P228	**Cuixia Chen** <cxchen@sdau.edu.cn>	*Genetic map-based cloning of two major QTL genes for husk number in maize (Zea Mays)*
P229	**Olamide Adesina** <oadesina@ksu.edu>	*Genetic mapping of resistance to Goss's wilt of maize*
P230	**Jacob Hinrichsen** <jhinrich@iastate.edu>	*Genome-wide dissection of leaf angle variation across the canopy in maize*
P231	**Diana Marcela Escamilla Sanchez** <descamil@iastate.edu>	*Genomic selection: Essence, applications and prospects*
P232	**Alain Charcosset** <alain.charcosset@inrae.fr>	*Genomic prediction and landscape genomics in a large maize landraces collection using high-throughput pool genotyping identifies promising sources of diversity for prebreeding*
P233	**Huyu Liu** <baixuefenfenxi@gmail.com>	*High intensity phenotyping sites: multi-scale, multi-modal sensing to identify the genetic regulation of plasticity/phenotypic stability.*
P234	**Eric Rodene** <eric.rodene@huskers.unl.edu>	*High-throughput phenotyping and machine-learning based approaches for time-series crop assessment using UAV imagery*
P235	**Phil Brailey-Jones** <pab14613@uga.edu>	*Host-specificities of microbial consortia revealed by metagenomic approaches in Sorghum bicolor*
P236	**Mercy Fakude** <mfakude@iastate.edu>	*Identification of genomic regions associated with the causal QTL of SHDG trait in Ames diversity panel by GWAS*
P237	**Hwi Moon** <hmoon3@ncsu.edu>	*Identification of waxy genotype from iodine-stained maize pollen image by linear discriminant analysis*
P238	**Joseph DeTemple** <detemplj@iastate.edu>	*Incorporating gene network information into the prediction of maize flowering time*
P239	**Beatrice Konadu** <bak234@cornell.edu>	*Introgression of Zea diploperennis to transfer perennial-like root N remobilization to maize (Zea mays)*
P240	**John Searl** <jsearl@wisc.edu>	*Kernel morphology influences maize hybrid seed production efficiency*
P241	**Collin Luebbert** <cluebbert@danforthcenter.org>	*Leveraging above and below ground phenotyping of a maize synthetic population containing teosinte alleles in nitrogen limited field conditions to identify nitrogen efficiency alleles*
P242	**Ramesh Kanna Mathivanan** <rmathivanan2@huskers.unl.edu>	*Linking known and previously uncharacterized genes to roles in maize metabolism and whole plant phenotype using paired transcriptomic and metabolic data from a field grown maize diversity panel*
P243	**Harel Bacher** <hb435@cornell.edu>	*Longitudinal modeling of stomatal conductance potentiates the optimization of water-use efficient maize*
P244	**Asher Hudson** <ahudson3@ncsu.edu>	*Maize hybrids display negative heterosis for induced defense while maintaining disease resistance*
P245	**Erik Ohlson** <erik.ohlson@usda.gov>	*Managing maize lethal necrosis disease using traditional and molecular breeding*

P246	**Mohamed El-Walid** <mze3@cornell.edu>	*Mapping freezing tolerance in tripsacum by bulk segregant analysis*
P247	**Alexander Mullens** <mullens3@illinois.edu>	*Mapping tissue-specific disease resistance to Xanthomonas vasicola pv. vasculorum in maize*
P248	**Matthew Wendt** <wendtmat@iastate.edu>	*Multi-environment multivariate genome wide association studies identify candidate loci underlying cuticular wax accumulation and composition on maize silks.*
P249	**Ty Thomas** <tsthoma2@ncsu.edu>	*Multi-environmental RNA-seq reveals the extent of genotype-by-environment interactions as it relates to gene expression.*
P250	**Robert Twohey III** <twohey2@illinois.edu>	*Optimizing a high-throughput screening method to identify the genetic regulators of VPD transpirational breakpoints in Zea mays*
P251	**Michael Burns** <burns756@umn.edu>	*Optimizing simulations for digital breeding that accurately parallels empirical data*
P252	**Sarah Jensen** <sarah.jensen@syngenta.com>	*Pangenome-based haplotype genomic prediction outperforms SNP-based prediction methods for North American corn breeding*
P253	**Tae-Chun Park** <tcpark@iastate.edu>	*Performance of triple cross hybrids with varying genetic distances among inbred parents*
P254	**Thomas Hartwig** <thartwig@mpipz.mpg.de>	*Phenotypic heritability in maize can be largely explained by genetic variation at transcription factor binding sites*
P255	**Jensina Davis** <jdavis132@huskers.unl.edu>	*Phenotypic plasticity in a maize hybrid population across a precipitation and elevation gradient spanning Iowa and Nebraska*
P256	**Dongdong Li** <ddli@iastate.edu>	*Phenotypic plasticity in flowering time in maize-teosinte introgression populations shaped by interaction between domestication loci and diurnal temperature range*
P257	**Denise E. Costich** <dc58@cornell.edu>	*Phenotypic selection of maize adapted to temperature extremes as a strategy to maintain productivity under global climate change*
P258	**Matthew Runyon** <mrunyon2@illinois.edu>	*Pipe dream or partitioning reality? Exploring extreme maize phenotypes and their role in programming senescence*
P259	**Harshita Mangal** <hmangal2@huskers.unl.edu>	*Profiling population level gene expression from a field grown sorghum diversity panel for comparative analysis with maize*
P260	**Waqar Ali** <wali3@huskers.unl.edu>	*Quantitative genetic analysis of variants contributing to variation in yield component traits in maize*
P261	**Justine Drouault** <justine.drouault@inrae.fr>	*Reconsidering the genetic diversity underlying photoperiod-sensitivity for maize adaption to climate change.*
P262	**Anna Giulini** <annapiamaria.giulini@crea.gov.it>	*Response of mature maize kernels to inoculation with Aspergillus flavus*
P263	**Ruairidh Sawers** <rjs6686@psu.edu>	*Response to arbuscular mycorrhizal fungi in field grown maize*
P264	**Diego Goncalves Caixeta** <caixeta@iastate.edu>	*Self-organizing maps for clustering tropical maize inbred lines cropped under low and high nitrogen inputs in Brazil*
P265	**Pearl Abu** <plabu@wacci.ug.edu.gh>	*SeqSNP-based genic markers revealed the genetic structure and identified candidate genes for low soil nitrogen tolerance in tropical quality protein (QPM) maize inbred lines*
P266	**Samantha Snodgrass** <snodgras@iastate.edu>	*Single parent expression and heterosis in a diverse diallel F1 population*
P267	**Lydia Keller** <lydiaekeller@gmail.com>	*Spatial analysis of stomatal patterning from computer vision-annotated images for GWAS/TWAS of a diverse biomass sorghum population*

P268 **Yingjie Xiao** <yxiao25@mail.hzau.edu.cn> — *Target-oriented prioritization: a breeding decision tool for genomic selection*

P269 **Seongmin Hong** <smhong.info@gmai.com> — *The core collection enriched for Korean maize (Zea mays L.) landraces reveals seed texture correlated morphological characters and novel multi-aleurone layer phenotypes*

P270 **Gen Xu** <gxu6@unl.edu> — *The dominance of gene expression controlled by trans-eQTLs contributes to heterosis of phenotypic traits in maize*

P271 **Chengxiu Wu** <cxwu@webmail.hzau.edu.cn> — *The maize ontogenetics: complexity, diversity and dynamics*

P272 **Aaron Kusmec** <amkusmec@iastate.edu> — *The many contexts of genetic effects*

P273 **Sendi Mejia Jimenez** <smejiaji@purdue.edu> — *The recessive lesion mimic mutation (les-2014) results from the loss of function of the only hpl1 gene in maize*

P274 **Jieli Wegerif** <jieliwegerif@ufl.edu> — *The role of Grassy Tillers 1 in drought response of Sorghum bicolor*

P275 **Randall Wisser** <randall.wisser@inrae.fr> — *The shape of data: a new concept for understanding crop diversity*

P276 **Jianming Yu** <jmyu@iastate.edu> — *To explain and to predict: Genetic diversity, environmental context, and phenotypic plasticity*

P277 **Karlene Negus** <knegus@iastate.edu> — *Transformers for genomic selection*

P278 **Christopher Mujjabi** <mujjabi2@illinois.edu> — *Unveiling the effects of organic nitrogen fertility and weed competition on corn hybrid agronomic performance and root architecture characteristics*

P279 **Max Braud** <mbraud@danforthcenter.org> — *Using remote and proximal sensing to quantify plant architectural variation across maize diversity*

P280 **Qiuyue Chen** <qchen295@wisc.edu> — *Weighted records prediction for hybrid maize performance*

Transposons & Epigenetics

P281 **Justin Scherer** <jts34805@uga.edu> — *Analysis of de novo mutator transposon insertions across tissue types*

P282 **Jonathan Cahn** <cahnjonathan@gmail.com> — *Bi-directionally expressed enhancers harbor the molecular signatures of maize domestication*

P283 **Kaitlin Higgins** <kaitlinj@iastate.edu> — *DNA glycosylase Mdr1 underlies a connection between DNA methylation and imprinted gene expression*

P284 **Samantha Jean** <jeans5@montclair.edu> — *Data mining dynamic transposable elements - Helitrons - using LSTM model*

P285 **Taylor Isles** <taylor.isles@outlook.com> — *Dynamic genomic evolution via active LTR transposable elements in maize*

P286 **Julian Somers** <jms53460@uga.edu> — *Exploring the timing and regulation of the sporophyte-to-gametophyte transition (SGT)*

P287 **Mateus Mondin** <mmondin@usp.br> — *Exploring tissue-specific variation in knob chromatin accessibility*

P288 **Manisha Munasinghe** <mmunasin@umn.edu> — *Genetic determinants of hybrid decay in backcross populations of maize*

P289 **Kempton Bryan** <kempton@uga.edu> — *Genomic consequences of repeated chromosomal breakage*

P290 **Mohammad Mahmood Hasan** <m.hasanbot@gmail.com> — *High-resolution crossover maps highlighting the role of DNA methylation in meiotic recombination of maize.*

P291 **Yirui Sun** <s.yirui@uga.edu> — *Influence of the DNA glycosylase MDR1 on histone modifications in endosperm*

P292 **Jason Lynn** <jlynn@cshl.edu> — *Maize AGO2 is involved in RNAi balance and epigenetic inheritance through the male germline*

P293 **Natasha Dhamrait** <ndhamrait@ucdavis.edu> — *Maize domestication through the lens of transposable elements*

P294 **Claire Menard** <menar060@umn.edu> — *TIPs and tricks for identifying transposable element insertion polymorphisms using short-read data*

P295 **Surinder Chopra** <sic3@psu.edu> — *The maize ufo1 and its mysterious function in kernel development*

P296 **Maike Stam** <m.e.stam@uva.nl> — *Vgt1: a cis-regulatory element regulating flowering time and growth speed*

P297 **Kathryn Koirtyohann** <kmk20cn@fsu.edu> — *mRNA splicing variation in mop1-1 mutant maize*

Evolution and Population Genetics

P298 **Ilksen Topcu** <ilksen.topcu@tamu.edu> — *Crop domestication in Zea mays has contrasting effects on microbiome community structure in above and below-ground microbiota*

P299 **Akwasi Yeboah** <akwasiyeboah97@gmail.com> — *Determination of genetic and epigenetic regulations of meiotic recombination under domestication in maize*

P300 **Madsen Sullivan** <madsens2@illinois.edu> — *Distribution and impact of functional glossy15 haplotypes on phase change, flowering, and grain drydown*

P301 **Wei-Yun Lai** <wl748@cornell.edu> — *Evolutionary mining of cold tolerance alleles in maize for future farming sustainability*

P302 **Heather Chamberlain-Irwin** <hchamber@iastate.edu> — *Genetic legacies of the first ancient South American State in archaeological maize*

P303 **Joseph Atemia** <j.atemia@fzjuelich.de> — *Genome-wide association studies to establish the genetic basis of agro-morphological and climatic factors in wild maize relatives*

P304 **Nicholas Gladman** <gladman@cshl.edu> — *Gramene 2024: A comparative resource on plant reference genomes, pan-genomes and pathways*

P305 **Senthilkumar Velmurugan** <senthilkumaranpbg12@gmail.com> — *Maize x teosinte synergy: A holistic vision for climate-resilient crop evolution through integrated innovative breeding strategies*

P306 **Elli Cryan** <epcryan@ucdavis.edu> — *Molecular evolution of a maize hybrid barrier over 12 million years suggests epistatic silencing*

P307 **Michelle Stitzer** <mcs368@cornell.edu> — *Revisiting the subgenome ancestry of maize*

P308 **Charles Hale** <coh22@cornell.edu> — *Scaling analyses of cis-regulatory evolution across hundreds of wild grass species*

P309 **Michael Anokye** <anomic17@gmail.com> — *Shoot-level phenotyping reveals adaptive variation in the grass genus Hordeum*

P310 **Rebecca Piri** <rdp22327@uga.edu> — *Tandem repeat structure and conservation in maize*

P311 **Jonathan Acosta** <jonathan.acosta@cinvestav.mx> — *The influence of heavy metal response in the origin and domestication of maize*

P312 **Aimee Schulz** <ajs692@cornell.edu> — *Uncovering the genetic basis of perenniality in the Andropogoneae*

P313 **Catherine Hardy-Ramey Li** <chli6@illinois.edu> — *Unraveling the genetic basis of kernel protein concentration in the Illinois long term selection experiment through near-isogenic lines*

P314 **Tyrus Cryan** <tycryan@davidson.edu> — *Phenotypic selection in SynAdapt, a genetically diverse maize-teosinte hybrid population*

Late Poster Submissions

P315 **Michael Scanlon** <mjs298@cornell.edu> — *Homology of the grass cotyledon: A multiplexed transcriptomic analysis*

P316 **Pyu Pyu Win** <pwin@hamilton.edu> — *Investigation of Zea mays Ab10 Kindr/Knob180 meiotic drive system in budding yeast*

P317 **Naima Akter** <nakter@hamilton.edu> — *Characterizing the kinetochore-microtubule binding interface and meiotic spindle structure of Zea mays*

P318 **Aniruddha Pathak** <anipath@iastate.edu> — *Regularized AMMI model for multi-environment agricultural trials*

Keynote Speaker Abstracts

Keynote Speaker 1 **Thursday, February 29 7:20 PM EST**

@cadwego

Phenomenology for maize experts: what do you see when you contemplate the map of global maize production?

(submitted by Ricardo Salvador <rsalvador@ucsusa.org>)
Full Author List: Salvador, Ricardo J[1]
[1] Union of Concerned Scientists; Washington DC, USA 20006

An agricultural system is the landscape expression of a set of human values, on the view of geographer Carl Sauer (1889 - 1975.) "The material manifestations of culture are land use, settlement patterns, technology, and other artifacts." The community of maize researchers and technologists has contributed to the creation of six global basins of agricultural hyper-productivity. The values manifested by the existence of these basins reflect prioritization of return to financial capital, industrial specialization and efficiency, at the expense of environmental degradation (global warming, soil erosion, hypoxia) and human displacement (decrease in the number, and migration, of both self-provisioning and industrial farmers.) To the extent that maize researchers intend their activities and contributions to be unqualified social goods, there is a need for this community to address the incongruity between those intentions and the actual social outcomes. The communities affected by the spread of industrial maize production, together with scientists whose expertise is understanding the grand currents and drivers of cultural development—anthropologists, historians, sociologists—can enrich the universe of considerations and evaluation of the "maize improvement" project.

Funding acknowledgement: Members of the Union of Concerned Scientists

Keynote Speaker 2 **Friday, March 17 11:40 AM EST**

Selected highlights of maize disease resistance genetics research: *These are a few of my favorite things.*

(submitted by Peter Balint-Kurti <pjbalint@ncsu.edu>)
Full Author List: Balint-Kurti, Peter J.[1]
[1] Plant Science Research Unit, USDA-ARS, Raleigh NC, USA

The maize genetic system has many unique and attractive features. While, perhaps surprisingly, it has not been used as much as some other systems in molecular-genetic studies of plant disease resistance, a number of important discoveries in this area have been made using maize.

The southern corn leaf blight epidemic of 1970, caused by the widespread distribution of (what turned out to be) a disease susceptibility gene, resulted in losses of ~$8 billion (2023 dollars). Maize pathologists were instrumental in helping ensure that the epidemic lasted just a single year. This epidemic has often been cited as a cautionary tale regarding the dangers of genetic uniformity. I will propose alternative perspectives and lessons that might be drawn from this event.

The first plant disease susceptibility and resistance genes were identified in maize. Studies of the maize *Rp1* common rust resistance locus were important in our understanding of the complex nature of many plant resistance loci. Further work with *Rp1* demonstrated the cell autonomous nature of the plant hypersensitive defense response (also known as HR). More recently, auto-active variants of *Rp1* have been used to describe the molecular genetic architecture that controls HR.

The excellent resources available in maize for quantitative genetic studies have enabled some of the most detailed studies describing the basis of quantitative disease resistance and multiple disease resistance. Several maize quantitative disease resistance genes have been identified over the past decade, informing our understanding of the complex basis of plant quantitative disease resistance.

I will discuss these 'selected highlights' with some reference to our own work. I will further discuss prospects and applications of our burgeoning knowledge in this area.

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

Keynote Speaker 3 **Friday, March 1 10:40 AM EST**

From the stars to your table - plants as complex conduits for iron nutrition

(submitted by Terri Long <terri_long@ncsu.edu>)
Full Author List: Long, Terri[1]
[1] Dept. of Plant and Microbial Biology, NC State University, Raleigh, NC 27695

Iron is a ubiquitous micronutrient that plays critical roles in central metabolic processes for all living organisms. The mechanisms by which plants extract iron from soil and maintain iron homeostasis are particularly intriguing. While it is relatively abundant, in most soils iron is insoluble and therefore of limited bioavailability, however excess iron accumulation in plants can lead to cellular damage. Thus, plants must extract sufficient quantities of iron from recalcitrant soil environments, while also ensuring that iron content does not exceed a specific range. Arabidopsis and other dicots have evolved mechanisms to sense iron deficiency in the shoot, which triggers roots to solubilize, reduce and uptake iron across multiple root cell types before transport to the shoot.

Using a combination of molecular and confocal microscopy analysis, cell-type specific transcriptional profiling, and mathematical modeling we have uncovered several molecular mechanisms that control how plants recognize and respond to iron deficiency stress in a root cell-specific manner. Our findings provide new evidence for how distinct alternations in the root cortex control carbon metabolism in response to iron deprivation, and how iron deficiency causes specific developmental alterations in the root vasculature and epidermis. Together, these mechanisms operate to fine-tune root growth and physiology in the face of suboptimal growth conditions, while also providing new avenues for exploring inter- and intracellular nutrient stress response in plants.

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

Keynote Speaker 4 **Saturday, March 2 10:40 AM EST**

Leveraging synthetic biology to monitor and control plant hormone activity

(submitted by Anna Stepanova <atstepan@ncsu.edu>)
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Phytohormones are critical regulators of plant development and environmental responses. In the past three decades, the molecular pathways that govern hormone biosynthesis, signaling, and catabolism have been largely mapped out using a combination of genetics, molecular biology, biochemistry, and cell biology approaches. Despite the major progress, our ability to monitor and precisely control hormone action remains limited. With the development of inexpensive DNA synthesis technologies and the rise of synthetic biology as a new discipline at the intersection of molecular genetics and engineering, new molecular tools can now be built to enable hormone tracking and targeted hormone manipulation. We have generated a synthetic biology toolbox that allows rapid construction of multi-hormone transcriptional reporters. In addition, we are building CRISPR-based logic gate devices to confer novel, highly restricted patterns of expression to any genes of interest using a limited set of available native and synthetic drivers. The latter technology can be employed to tune the expression levels and subtract undesired domains of expression from existing drivers to precisely control output genes of interest, such as hormone biosynthesis, signaling, or catabolism genes, to regulate plant architecture, responses to stress, and other traits of interest. By combining multi-hormone reporters and genetic logic devices, we aim to shed fresh light on the mechanistic role of hormones in orchestrating plant development and stress physiology. That knowledge can then be relied upon to develop resilient next-generation crops.

Funding acknowledgement: National Science Foundation (NSF)

Keynote Speaker 5 **Saturday, March 2 11:40 AM EST**

Plant breeding and the infinitesimal model: cause or consequence

(submitted by Natalia de Leon <ndeleongatti@wisc.edu>)
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Breeding relies on genetic variation generated through random mutagenesis and recombination and simultaneously shapes such variation by the process of selection. Starting with the domestication processes which led to the development of modern crops, to contemporary tools that refine artificial selection, large beneficial effects controlling targets of selection are expected to be fixed, limiting subsequent improvements to genes of minor effect. This limitation, however, does not result from biological constraints but rather from the allelic purge induced by selection. The practical consequence of this is that enhancing economically important traits in plants exclusively through the exploration of existing variation is unlikely to yield significant progress, particularly at the scale demanded by a growing global population. The advent of modern technologies, including genotype-agnostic transformation and editing resources, enable breeders to move away from a traditional breeding lottery approach to a situation where it is now feasible to deliberately optimize combination of alleles that can be further exploiting from a breeding context. As we consider sources of variation and methods to evaluated and harness them effectively, it is important to consider where such variation is prevalent and which techniques hold promise for generating the magnitude of changes required to meet global demand. This presentation will discuss how selection processes have likely altered variation and the ramifications for technological applications aimed at increasing the efficiency of breeding efforts.

Funding acknowledgement: United States Department of Agriculture (USDA), National Science Foundation (NSF), Foundation for Food & Agricultural Research (FFAR), National Corn Growers Association (NCGA), Iowa Corn Growers Association (ICGA)

McClintock Prize Abstract

McClintock Prize (M1) **Friday, March 1 11:40 AM EST**

Polycomb memory to register environmental exposure

(submitted by Caroline Dean <caroline.dean@jic.ac.uk>)
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Plants are completely tuned to their environment, changing their metabolism and growth in response to short-term changes and their development in response to long-term trends. A question that fascinated Barbara McClintock was how the environment affected plant genome functioning, but this is still relatively poorly understood. We have addressed this question by studying how naturally fluctuating temperature signals are registered throughout winter in the process of vernalization, a process that aligns flowering with favourable spring conditions. In Arabidopsis, vernalization involves the epigenetic silencing of the floral repressor *FLC.* At its simplest this a cold-induced Polycomb silencing mechanism showing many parallels to Polycomb regulation generally. However, further dissection has shown that multiple aspects of the noisy temperature signals influence many processes distributed throughout the *FLC* regulatory network. These function directly through co-transcriptional changes of *FLC* sense and antisense transcription, and indirectly through reduced cell division. These temperature-dependent inputs are integrated through promotion of a low probability Polycomb-mediated chromatin switch. This occurs locally at each allele and holds the memory of that temperature exposure through the rest of development. The talk will describe our understanding of this environmentally mediated epigenetic switching mechanism.

Funding acknowledgement: Royal Society, BBSRC, Wellcome, ERC

Short Talk Abstracts

SESSION 2 – THE GENES THAT MAKE MAIZE
Chair: Madeleine Bartlett Friday, March 1. 8:00 AM – 10:10 AM EST

T1

An exocyst component ZmEXO70F4 coordinates with jasmonate signaling pathway to regulate maize immunity to Gibberella stalk rot

(submitted by Xiquan Gao <xgao@njau.edu.cn>)
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Gibberella Stalk Rot (GSR) caused by *Fusarium graminearum* is one of the most devastating diseases worldwide, which significantly reduces the production and quality, and restricts the application of mechanic harvesting technology in maize. Despite that several resistance genes associated with GSR have been cloned, more candidate genes need to be characterized, and the complex genetic and molecular mechanisms underlying GSR immunity await to be fully dissected, considering that the trait is controlled by many minor-effect loci. We identified a novel GSR-resistant QTN *qRGS1*, and *ZmExo70F4* was identified as the candidate gene via Genome-wide association study (GWAS), allelic analysis, and gene functional analysis. Using yeast-two hybrid library screening, combined with RNA-seq and WGCNA, multiple immune signaling components, including those from jasmonates signaling pathways, were identified to be associated with *ZmExo70F4*-meidated immunity to GSR. The interactions between ZmEXO70F4 and its potential interacting proteins including ZmMYC7 was confirmed and their functions in GSR immunity were validated. ZmMYC7 was further found to modulate GSR immunity through regulating isoflavonoids production. The results obtained will not only help us to deep insights into the complex GSR resistance mechanisms, but also provide novel genetic resources for molecular breeding of maize stalk rot resistance.

Funding acknowledgement: JBGS [2021-002] project from the Government of Jiangsu Province, the National Key Research and Development Program of China (grant #: 2020YFE0202900), Beijing Lantron Seed CO., LTD

T2

Crazy leaves and truffula trees

(submitted by Annis Richardson <annis.richardson@ed.ac.uk>)
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Plant architecture is crucial in determining productivity. In maize the iterative development of units called phytomers; composed of a node, internode, axillary meristem and organ such as a leaf; defines final plant shape. Modulation of the growth and development of each part of the phytomer also contributes to morphological diversity underpinning differences in productivity. Auxin plays a key role in regulating phytomer development, however the pathway that decodes its effects is not well understood, in part due to few reported auxin mutants with vegetative phenotypes. Here we present two dominant maize mutants with opposing vegetative phenotypes; *Hoja loca1*(*Oja*) and *Truffula*(*Trf*); both of which have defects in auxin transcriptional response. *Oja* plants often fail to produce leaves at each node, and those that do form can be midribless or tube-shaped. *Oja* is due to a mutation in the highly conserved degron motif of the auxin response repressor ZmIAA28. In contrast, *Trf* plants develop extra leaves at nodes, which can have multiple midribs. The *Trf* mutation is located in the DNA-binding domain of ZmARF28, a member of the repressive class-B Auxin Response Factor clade that we know relatively little about. Our unique comparative analysis of maize and moss, species separated by ~500 million years of evolution, reveals a novel evolutionarily conserved degron motif, shedding light on a new pathway for class-B ARF regulation in plants. Through investigating *Oja* and *Trf* we not only build new understanding of auxin-regulated phytomer development in maize, but also uncover fundamental components of auxin response regulation important for land plants.

Gene / Gene Models described: *Oja (ZmIAA28), Trf (ZmARF28)*; GRMZM2G035465, Zm00001eb408800

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

T3

A thiamin pyrophosphate kinase encoding gene *ZmTDPK2* finely regulates ear length and grain yield in maize

(submitted by Yun Luo <1332803470@qq.com>)
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Enhancing the grain yield of maize is imperative to meet the escalating demands for maize-derived products in food, feed, and fuel. Ear length (EL) serves as a crucial determinant of grain yield and, consequently, stands as a pivotal target trait in maize breeding. However, only a limited number of EL quantitative trait loci (QTLs) have been identified and cloned, with their underlying molecular mechanisms remaining largely elusive. Here, we characterize an EL QTL, *qKB6.2*, to identify a maize gene controlling flower number and ear length. The *qKB6.2* encodes a thiamin diphosphokinase2 (*TDPK2*), a gene that involves phosphorylating thiamin into thiamin pyrophosphate (TPP) in the cytoplasm. CRISPR/Cas9 knockout lines of *ZmTDPK2* exhibited lower TPP levels in the developing ear, reduced florets number, EL and thus decreased grain yield. Interesting, the maize EL of *ZmTDPK2* overexpression lines exhibited a trend of initially increasing and then decreasing with the rising expression levels of *ZmTDPK2*, suggesting that the optimal EL is manifested at the appropriate *ZmTDPK2* expression levels. The favorable haplotype frequency of *qKB6.2* in the association population is only 0.4%, which is rare. And, in comparison with the hybrid lines constructed with NIL$^{qkb6.2}$, the EL and grain yield of hybrid lines constructed with favorable haplotype NIL$^{qKB6.2}$, increased by 6.3%-17.4%, and 5.5%-17.4%, respectively. Moreover, foliar application of a 0.04% TPP solution on the leaf surface has been shown to elevate the yield of maize, rape and rice. Our findings suggest that *ZmTDPK2* serves as a critical role in TPP-mediated female inflorescence development, EL and grain yield, and also provide a tool to improve grain productivity by optimizing *ZmTDPK2* expression levels or foliar application of TPP in other cereals.

Gene / Gene Models described: *ZmTDPK2*; Zm00001d037916
Funding acknowledgement: National Natural Science Foudation of China and China Postdoctoral Science Foundation

T4

A novel domestication gene establishes developmental boundaries

(submitted by George Chuck <georgechuck@berkeley.edu>)
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Early farmers selected for a suite of different vegetative and reproductive crop traits during domestication but identifying the causative loci has been hampered by their epistasis and functional redundancy. Using ChIP-seq combined with genome wide association analysis, we uncovered a developmental regulator that controls both categories of traits while acting upstream of multiple domestication loci. Mapping of four maize domestication and improvement QTL identified *tasselsheath4* (*tsh4*) as a strong candidate gene. We show that *tsh4* functions redundantly with a pair of homologs to bind and activate three domestication genes, *teosinte branched1*, *tassels replace upper ears1* and *teosinte glume architecture1*. *tsh4* is in fact a novel domestication gene that establishes developmental boundaries and specifies meristem versus lateral organ fates. TSH4 does this by employing an unusual mechanism where it targets and represses the very same microRNAs that negatively regulate it via a double negative feedback loop. TSH4 then cements these boundaries by binding to *AUX IAA* genes to repress auxin responsiveness in lateral organs and redirect the flow of auxin to axillary meristems to enable their growth. One such meristem is the spikelet pair meristem that is responsible for doubling seed yield in maize versus teosinte and is missing in higher order *tsh4* mutants. A comparison of the maize versus teosinte alleles of *tsh4* within introgressed backgrounds revealed that the former contains a small intron deletion and is more highly expressed. Thus, by selecting for this high expressing allele early farmers were able to modify a variety of key architectural traits to maximize yield gain, placing *tsh4* at the top of the domestication hierarchy.

Gene / Gene Models described: *tsh4, ub2, ub3, tb1, tru1, tga1*; Zm00001eb316740, Zm00001eb035030, Zm00001eb199880, Zm00001eb054440, Zm00001eb141160, Zm00001eb175150

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

T5

Leaf angle and curricular innovation: a CURE for research and undergraduate education

(submitted by Mark Lubkowitz <mlubkowitz@smcvt.edu>)
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Undergraduate research is arguably the single most important experience for inspiring students to pursue graduate school and STEM careers. Yet, most students never have the opportunity to engage in research simply because of time and limited institutional resources. Course Based Undergraduate Research Experiences (CUREs) address these issues since students and institutions are already committed to classes. CUREs are not new to undergraduate curricula but they often occur as a singular opportunity as opposed to a curricular foundation, and therefore lack the impact of a prolonged research experience. To address this issue, we have developed a four-year CURE program that consists of eleven courses spanning four STEM majors. Students experience a breadth of research systems, questions, approaches, and analyses at every developmental level and the scale of the program ensures that a significant portion of every student's education is research based. Here we present an overview of our CURE program and then illustrate how undergraduate researchers are helping to identify transcription factors that control leaf angle in maize. Leaf angle is an important trait that has historically been manipulated by breeders to increase planting density and therefore yield. Since the auricle contributes significantly to leaf angle, undergraduates helped perform a yeast one hybrid screen using the promoters of *narrowsheath1*, *liguleless1*, *liguless3*, and *roughsheath1;* all genes that are expressed in the pre-ligular band during leaf development. Surprisingly, all of these genes were bound by some combination of only four transcription factors in the Lateral Organ Boundary (LOB) family, including *ramosa2*. The small number of transcription factors identified as well as the overlap suggests that these four genes are in the same regulatory network and function early in auricle development. This finding illustrates how undergraduate education, CUREs, and discovery can be joined to the benefit of all parties.

Gene / Gene Models described: *ns1, lg1, lg3, rs1, ra2*; GRMZM2G069028, Zm00001eb067740, GRMZM2G087741, GRMZM2G087741, Zm00001eb299420, Zm00001eb299420

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

T6

Optimized *ZmWOX2A*-based transformation system enhances somatic embryogenesis, transformation efficiency and gene editing efficiency in maize (*Zea mays* L.) and sorghum (*Sorghum bicolor* L.)

(submitted by Jonathan Matheka <matheka@wisc.edu>)
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Embryogenic cultures are key components of many maize genome engineering/editing systems. Production of embryogenic cultures is, however, highly genotype dependent. Previously, we identified an embryogenesis-related maize gene, *ZmWOX2A*, and demonstrated that when constitutively expressed it enabled somatic embryogenesis and regeneration of phenotypically normal plants of the recalcitrant maize genotype, B73. Initial comparisons with a *BBM+WUS*-based system showed that the constitutive *ZmWOX2A* system generally resulted in lower somatic embryogenesis and transformation frequencies (TF). To optimize the *ZmWOX2A*-based system, the effects of 6 terminators, 6 promoters, and 6 enhancer-containing introns were evaluated as components of the *ZmWOX2A* transcriptional unit (TU). Of the 6 termination sequences tested, the tobacco extensin terminator (*EU*t) significantly increased *ZmWOX2A*-induced somatic embryogenesis (SE) and was used in TUs testing promoter and intron effects. The promoters driving expression of *ZmWOX2A* that were tested for effects on TF and plant phenotype included constitutive (*ZmUbi1*p), scutella epidermis-specific (*PLTP*p), and 4 novel, embryo-specific promoters we recently cloned and characterized. Significantly more somatic embryos were produced per explant with the *ZmUbi1*p, relative to *PLTP*p; however, there was no significant difference in TFs between the *ZmUbi1*p- or *PLTP*p-driven *ZmWOX2A/EUt* constructs. Two of the four embryo-specific promoters yielded efficient TFs, but below that of either the *ZmUbi1* or *PLTP* promoters. The highest performing embryo-specific promoter (*Php20719a*) was then paired in 6 intron combinations to compare effects on TF in the *ZmWOX2A* system. Three of the introns significantly increased TF of the *Php20719a*p-containing TUs more than that of the unmodified *Php20719a*p, two of which also outperformed *PLTP*p. Optimized *ZmWOX2A* TUs were then tested on several maize and sorghum genotypes and efficient TFs achieved in both species. Optimized vectors were also tested in maize editing experiments and resulted in efficient generation of edited, phenotypically normal plants and progeny.

Funding acknowledgement: National Institute of Food and Agriculture (NIFA)-United States Department of Agriculture (USDA)-Hatch grants #1020442 and 1013262, and National Science Foundation (NSF)-IOS grant # 1917138

T7

Harnessing AI for agricultural innovation

(submitted by Steve Johnson <steven.johnson1@bayer.com>)
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As the depth and complexity of scientific data and literature expand, the role of artificial intelligence (AI) has become increasingly pivotal. AI techniques are proving to be an indispensable tool for sifting through and integrating disparate streams of information, paving the way for unprecedented breakthroughs. From protein design to cell fate determination, there are a growing number of examples of AI technologies providing key contributions to scientific discovery. Bayer Crop Science is fully engaged in the pursuit of cutting-edge solutions for the next wave of maize agricultural production. To achieve this, we have adopted a multi-level strategy that harnesses the latest developments in AI technology. In this session, we will shed light on how Bayer Crop Science is leveraging AI for large-scale yield prediction, redefining phenotyping with computer vision techniques, and augmenting the productivity of individual scientists. Furthermore, we will unveil our approach to Machine Learning Operations (MLOps), which plays a crucial role in sustaining and advancing the complex array of AI models that are integral to our product development pipeline. This talk aims to provide insights into the multi-level, dynamic contributions AI can play in modern agricultural research.

T8

A practical haplotype graph map of the ZeaSynthetic population enables integration of teosinte alleles into breeding efforts

(submitted by Micah Kelleher <mkelleher@danforthcenter.org>)
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Domestication, adaptation, and inbreeding of modern maize has resulted in a significant loss of the genetic diversity found in the wild species of maize, teosinte. Genetic variation in teosinte could provide a useful source of diversity lacking in modern maize cultivars. However, the lack of high-resolution genetic resources for the study of teosinte has made it difficult to associate that variation with agronomic traits. We produced a genetic map of the Zea Synthetic population, a genetic resource created by randomly mating a 38-accession synthetic breeding population consisting of 11 geographically diverse teosinte (Zea mays ssp. parviglumis), B73, Mo17, and the 25 inbred Nested Association Mapping (NAM) founders. After 6 generations of random mating, 2000 DHs were produced, originally to study inbreeding depression.Our approach leverages full genome sequences of the population's parent genomes using the practical haplotype graph (PHG), a server mounted pangenome built from a SQL database populated by a reference panel of whole genome sequences. To mirror the makeup of our Zea Synthetic population, we filled a reference panel with data from the whole-genome sequences of B73, Mo17, the 25 inbred NAM founders, and 11 teosinte pseudo-genomes. Approximately 4.6 million haplotypes were sampled from individuals and groups in this diverse panel to populate the PHG with individual and consensus reference haplotypes. These haplotypes were paired with low coverage GBS sequences from the 2000 DHs to accurately impute WGS data for the entire population for use in association mapping. Our map is able to identify QTL associations for traits such as flowering time and Cadmium accumulation as well as identify regions of segregation distortion and those that have experienced selection.

Funding acknowledgement: United States Department of Agriculture (USDA), Donald Danforth Plant Science Center

T9

Cyto-swapping in maize by haploid induction with a *cenh3* mutant

(submitted by Esteban Bortiri <esteban.bortiri@syngenta.com>)
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Modern agriculture is increasingly reliant on traited hybrid maize because of its superior yield, resistance to insect damage, and ease of weed control. Detasseling of female line plants during commercial seed production costs millions of dollars annually and lowers seed yield. The use of Cytoplasmic Male Sterility (CMS) avoids detasseling and decreases the cost of hybrid seed. However, conversion of female maize lines to CMS involves several rounds of backcrosses after the initial cross to a CMS donor line to reconstitute the traited female Recurrent Parent (RP) with minimal linkage drag. This process can take up to 2 years and results in approximately 95% Recurrent Parent (RP) recovery. Maize mutants of the *centromeric histone H3* (*cenH3*) gene can form haploids that inherit only chromosomes of the pollinating parent but the cytoplasm from the female parent. We successfully used a *cenH3* mutant to cyto-swap seven Non-Stiff Stalk elite lines by using them as pollen donors to *cenH3* mutant ears, identifying haploids by genotyping, and doubling their genome. The rate of haploids recovered was 5% and genotyping with a large panel of markers confirmed the Double Haploids are 100% RP. We created a *cenH3* mutant line that carries the dominant embryo color marker *R1-scm2* and a CMS-C cytoplasm to perform conversions to CMS by cyto-swapping. This line was used to convert nine Stiff Stalk and four sweet corn lines to CMS in under 10 months, averaging 5% of haploids in the F1. The lines converted to CMS by cyto-swapping are 100% isogenic to RP and have no genome from the conversion line. The conversion line has no transgenic elements and has been declared exempt from regulation status by the USDA. We envision that cyto-swapping with *cenH3* will be applicable in CMS deployment in diverse crops to create superior hybrid varieties.

Gene / Gene Models described: *cenH3, R1-scm2*; Zm00001eb291210, Zm00001eb429330

T10

A foundational large language model for edible plant genomes provides accurate predictions of regulatory elements and gene expression in the maize genome

(submitted by Javier Mendoza Revilla <j.mendoza@instadeep.com>)
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Significant progress has been made in the field of plant genomics in recent years, especially through the increased use of high-throughput methodologies that characterize multiple genome-wide molecular phenotypes. Given the time and cost of performing these experiments, a critical step for more efficient crop improvement is effectively leveraging them to make accurate predictions from genomic sequences alone.
We present a novel foundational large language model, the Agronomic Nucleotide Transformer (AgroNT), based on the transformer architecture and pre-trained on reference genomes from 48 edible plant species. To explore how the AgroNT performs on predictions from genomic sequences alone, we further fine-tune it with genome-wide high-throughput experiments and compare performance to other available models. In multiple prediction tasks and across diverse plant species, we show that AgroNT obtains state-of-the-art predictions for many genomic elements in multiple plant genomes.
In maize, we apply the AgroNT to multiple prediction tasks related to gene regulation. We have shown it has high performance (AUC = 0.99) in classifying maize transcripts as protein-coding (mRNA) or potentially regulatory (lncRNAs). Leveraging Assay for Transposase-Accessible Chromatin with high-throughput sequencing (ATAC-seq), it also classifies chromatin regions as open with high accuracy (AUC = 0.98). Next, we explored AgroNT's ability to make numerical predictions from promoter sequences. First, we predict promoter strength as measured by Self-Transcribing Active Regulatory Region sequencing (STARR-seq) and obtain high correlation between observed and predicted values (average R2 0.73). We also use in vivo RNA-seq experiments to predict gene expression levels across 23 diverse maize tissues and show that our predictions correlate well with experimentally measured values (average R2 0.62).
These results show that our foundational large language model has learned functional genomic features and can accelerate trait research via discovery of gene regulatory features from sequence alone, potentially accelerating efforts in genomic improvement.

T11 @JeanMichelAne

Biological nitrogen gixation on the aerial roots of maize for sustainable agriculture

(submitted by Jean-Michel Ane <jeanmichel.ane@wisc.edu>)
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Maize (Zea mays L.) is an important crop due to its high yield and versatility, contributing significantly to global food security and economic stability. We have shown that some maize landraces from southern Mexico obtain 29–82% of their nitrogen from the atmosphere by hosting nitrogen-fixing (diazotrophic) communities in a mucilage produced by their aerial roots after rain. The maize mucilage is produced by border cells that detach from the aerial root cap in a process similar to the underground root cap. However, border cells are much more abundant and larger in the mucilage from aerial roots than those produced by underground roots. We characterized the aerial root development of several maize landraces and showed the effect of both genetic and environmental factors. A quantitative trait loci approach was conducted in biparental and doubled haploid populations to identify plant genes controlling the number of nodes with aerial roots and their thickness. Humidity and soil nitrogen also affect aerial root development. We successfully isolated over 150 distinct diazotrophs from maize mucilage, developed non-fixing mutants as controls, and enhanced ammonium-excreting mutants from some of these isolates. The distinct symbiotic relationship of diazotrophs with maize aerial roots offers a promising avenue for utilizing biological nitrogen fixation to reduce reliance on synthetic fertilizers and promote more productive and sustainable agricultural practices.

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

T12

Decoding nitrogen use efficiency in maize and sorghum: Insights from comparative gene regulatory networks for sustainable agriculture

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The dual role of nitrogen in plant growth is essential yet potentially harmful in excess, posing challenges for agricultural productivity and environmental sustainability. Understanding plant adaptation to varying nitrogen levels is crucial for developing crops with improved nitrogen use efficiency (NUE). This study aimed to: 1) identify conserved regulatory architectures between dicots and monocots using Yeast One-Hybrid (Y1H) Gene Regulatory Networks (GRNs), and 2) characterize subnetworks within these GRNs related to nitrogen limitation and recovery in maize and sorghum. We constructed a maize-specific GRN, comprising 1625 Protein-DNA Interactions (PDIs) with 70 promoters and 301 transcription factors (TFs), and compared it to an existing Arabidopsis GRN. Our analysis revealed that 18% of the conserved interactions in the Arabidopsis GRN were matched by 11% in maize, indicating weaker conservation among transporter genes but significant conservation in the nitrate assimilation pathway (38 PDIs in Arabidopsis, 65 PDIs in maize). The bZIP family of transcription factors emerged as key regulators in maize, especially bZIP18/bZIP30, which demonstrated substantial outdegree interactions and feed-forward loops. Under hydroponic conditions with varying ammonium nitrate concentrations, maize and sorghum plants were grown and their leaf and root tissues sampled for RNA-seq analysis. Both species exhibited variable proportions of differentially expressed genes (DEGs) across different time points and tissues. In maize, DEGs peaked at 38% in leaves after 3 hours and 27% in roots during the 24.5-hour recovery phase. Sorghum displayed 35% of genes differentially expressed in leaves at 3 hours and 27% in roots at 24.5 hours. Among various transcription factors, maize and sorghum showed divergent expression patterns, particularly within the bZIP family and NIN-LIKE PROTEIN (NLP) transcription factors known for nitrogen regulation. This study illuminates complex genomic regulatory frameworks underpinning plant adaptability to fluctuating nitrogen levels, providing valuable insights for selecting genotypes with enhanced NUE, contributing to sustainable agricultural practices. This project was funded by the USDA-ARS award number 8062-21000-044-000D.

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T13

The nitrogen cycle in the rhizosphere of diverse grass species in Andropogoneae

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Nitrogen (N) fertilization in modern crop production has a profound impact on the N cycle in the ecosystem. Excess N in the soil leads to nitrous oxide (N2O) emissions and to nitrate (NO3-) leaching. Breeding for efficient N use and recycling in a cropping system is crucial for global sustainability. An optimized strategy for N reuse may not exist in modern crops given the lack of selection and strong bottleneck during domestication. However, we hypothesize their wild relatives may keep the favorable phenotypes. Hence, we examined the N cycle in the rhizosphere of 39 grass species related to maize and sorghum. We discovered significant phylogenetic variation in potential nitrification rates and NO3- loss in the rhizosphere soil of diverse species. Contrary to our hypothesis, all annual species including sorghum and maize are amongst the N “Conservationists,” inhibiting nitrification and conserving N within the system. Many perennials are either "Leachers" that enhanced nitrification and leaching or "Nitrate Keepers" with similar nitrification enhancing effect but lost less NO3-. Although this result rejects our hypothesis, it unveils the diverse abilities to influence soil microbial chemistry and the varying N use efficiencies of grass species. We investigated the native habitat environments of the studied species and showed that several soil characteristics including acidity and fertility have been the key drivers shaping the plant-microbe interaction in the rhizosphere. Using comparative genomic and transcriptomic approaches, we nominated key genes encoding transporters for inorganic N and secondary metabolites that underlies the phylogenetic variation in rhizosphere N cycle. We anticipate these genes could be harnessed to improve agricultural sustainability with further functional studies.

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T14 @spklein2

Genomic structural variation underlies differential root responses to nitrogen stress in maize

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Uncovering the functional relationships between genome and phenome are critical for efficiently selecting desirable traits for plant resilience to environmental stress. Unlike genomic prediction that uses markers to link genotype to phenotype, whole genome assemblies can be used to predict phenotypic outcomes based on structural variation in genic and the vast intergenic regions. Using the maize Nested Association Mapping (NAM) population, we sought to identify structural variants associated with root growth angle (RGA), a root architectural trait that is important for nitrogen (N) acquisition and plastic in low N environments. Gene candidates contributing to RGA have been recently detected, but the genetic control of its plasticity is poorly understood. We surveyed the NAM founders in the field under high and low N and identified two genotypes that warranted further investigation because of their contrasting root responses to low N: B73 (steep-angled and non-responsive to stress) and Oh7B (whose RGA became significantly steeper under low N). In a followup field study, we observed a wide range of RGA plasticity in response to low N in the NAM family of recombinant inbred lines derived from B73 and Oh7B. Using linkage mapping, we identified genomic regions associated with RGA plasticity and evaluated these regions further by developing a tool to compare genomic structural variation between B73 and Oh7B. To bridge the gap between phenotypes and genome regions, we also assessed differential transcript abundance within these regions using RNAseq to identify specific elements that could account for these differential phenotypic responses to low N. Our results demonstrate that we can identify new breeding targets for increased resiliency by using a multiomics approach.

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T15

Mining ancient genomes for microbiome associated phenotypes for nutrient retention in agroecosystems

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Plant-microbe interactions in the rhizosphere govern availability of nutrients and are a target for improving crop sustainability. However, the ability of modern crops to recruit and structure their microbiome has been altered by domestication and breeding. Selection for crop plants based on aboveground traits in high nutrient environments has inadvertently led to changes to belowground plant physiology and relationships with the soil microbiome, altering microbiome functions that contribute to sustainability and environmental quality (e.g. nutrient acquisition, nutrient retention, and GHG production). We hypothesized that plant genotypes differ in their ability to recruit microbial functional groups, and ultimately that the functional profile of the microbial community can be treated as a selectable plant phenotype and optimized through plant breeding. We surveyed the rhizosphere microbiome of diverse maize genotypes to compare their capability to recruit microbial nitrogen cycling functional groups and examined rates of nitrogen transformations. Significantly different N cycling microbial communities were observed among maize genotypes that represent the endpoints of directed evolution, as well as among germplasm selected under different levels of fertilization. Our results showed significantly lower rates of nitrification and denitrification in ancestral lineages of maize (teosinte). Maize-teosinte near isogenic lines were used to narrow down the genetic region associated with these nutrient retention traits to explore the mechanistic basis for these microbiome-associated phenotypes. We explored the combinatorial effect of plant-microbe interactions for their ability to both provision (via N fixation) and retain nutrients, which resulted in elevated nutrient acquisition by the crop as well as sustainable environmental outcomes. Our results link host-associated microbiome and ecosystem function, and demonstrate the genetic capacity to optimize recruitment of N-cycling functional groups and improve crop sustainability. Identifying microbiome-associated phenotypes will allow breeders and ecosystem scientists to select crop cultivars that improve the efficiency and sustainability of agriculture and protect environmental quality.

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T16

Teosinte pollen drive guides maize diversification and domestication by RNAi

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Meiotic drivers subvert Mendelian expectations by manipulating reproductive development to bias their own transmission. Chromosomal drive typically functions in asymmetric female meiosis, while gene drive is normally postmeiotic and typically found in males. Using single molecule and single-pollen genome sequencing, we describe *Teosinte Pollen Drive*, an instance of gene drive in hybrids between maize (*Zea mays ssp. mays*) and teosinte *mexicana* (*Zea mays ssp. mexicana*), that depends on RNA interference (RNAi). 22nt small RNAs from a non-coding RNA hairpin in *mexicana* depend on *Dicer-Like 2 (Dcl2)* and target *Teosinte Drive Responder 1 (Tdr1),* which encodes a lipase required for pollen viability. *Dcl2*, *Tdr1*, and the hairpin are in tight pseudolinkage on chromosome 5, but only when transmitted through the male. Introgression of *mexicana* into early cultivated maize is thought to have been critical to its geographical dispersal throughout the Americas, and a tightly linked inversion in *mexicana* spans a major domestication sweep in modern maize. A survey of maize landraces and sympatric populations of teosinte *mexicana* reveals correlated patterns of admixture among unlinked genes required for RNAi on at least 4 chromosomes that are also subject to gene drive in pollen from synthetic hybrids. *Teosinte Pollen Drive* likely played a major role in maize domestication and diversification, and offers an explanation for the widespread abundance of "self" small RNAs in the germlines of plants and animals.

Gene / Gene Models described: *tdr1 GRMZM2G390678*; Dcl2 (Dcl105) GRMZM2G301405

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T17

How recombination facilitated the domestication of maize.

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Since maize was domesticated 10,000 years ago, its genome composition has been radically altered. Although selection during domestication reduced the genetic diversity of maize, meiotic recombination counteracted this reduction through the breakage of linkages between loci. Therefore, recombination has played a major role in shaping the genomic landscape of modern maize. It has been hypothesized that domestication increases global recombination rates. However, the role of recombination during domestication has not been thoroughly investigated and the intricate relationship between the recombination landscape and maize domestication has not been examined. Studying this phenomenon on a fine scale requires large high-resolution recombination maps, which do not exist in plants. To explore the maize recombination landscape during domestication, we inferred past recombination events through the population genomics method of coalescence, which is a backward-in-time approach to reconstruct a population's genealogy. We also used an identity-by-descent analysis, which identifies shared haplotype blocks. Together, these approaches yielded one million recombination events enabling us to construct the first high-resolution maps of recombination rates in plants. These unparalleled maps permitted us to examine the variation between the maize and teosinte recombination landscapes. First, we were able to confirm domestication increased the global recombination rate in maize (0.71 cM/Mb) compared to teosinte (0.63 cM/Mb). We also found that maize recombination rates were additionally increased at the boundaries of domestication selective sweeps, suggesting that recombination increased the efficiency of selection. To understand mechanistically how recombination rates evolved, we investigated recombination genes in maize and teosinte. We found selection signatures in recombination genes in maize and identified some of those genes to be either candidates for direct selection targets or located within domestication selective sweeps. We propose that selection during domestication affected recombination patterns which in turn made selection more efficient.

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T18

Maize development is controlled by an ancient nutrient signaling pathway

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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 study the impact of changing nutrient availability on maize development, we designed a nutrient-limiting assay with sixteen unique conditions 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. Combined with phenotypic and targeted molecular analyses, these experiments demonstrate that TOR precisely responds to changes in sugar availability by regulating gene expression at every level—transcriptionally, post-transcriptionally, translationally, and post-translationally. We have identified thousands of DEGs, hundreds differentially abundant metabolites, and dozens of phosphorylation events all dependent on TOR's response to nutrient levels. More specifically, we have identified deeply conserved residues in TOR targets that are identical across 150 million years of evolution. We have also identified novel targets and networks in maize related to C4 photosynthesis and grass developmental patterning. Together, these data provide a map for future genetic interventions to enhance nutrient use efficiency in maize.

Gene / Gene Models described: *tor1*; Zm00001eb285840

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T19 @snodgrasshopper

Fractionation in the tripsacinae subtribe

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Polyploidy and fractionation— the processes by which genomes grow and shrink in size—characterize plant genome evolution. The rate of fractionation has typically been studied using a single species relative to a diploid reference or a set of species each descendent from different whole genome duplication events (WGD). From these, fractionation has been hypothesized to occur quickly after the initial WGD event and the genome re-diploidized before subsequent speciation events. The ancestral species of the Tripsacinae subtribe, which contains the genera Tripsacum and Zea, underwent a WGD ~12MYA after divergence from Sorghum. The descendant species that comprise the subtribe, including maize, have since fractionated back to a diploid-like state with Zea genomes comprised of 10 chromosomes and Tripsacum 18. Previous work in maize demonstrate ongoing fractionation at the timescale of hundreds of years, much more recent than earlier hypothesized. To better estimate the timing and rate of fractionation, we use high-quality, de novo assemblies of genomes across the Tripsacinae. We developed a pipeline to accurately identify shared and segregating fractionation of homoeologs across 35 Tripsacinae genomes. Using parsimony, the timing of fractionation is estimated across the clade. Preliminary findings suggest that, while most fractionation is shared across all genomes and occurred before the divergence of genera or right after, Tripsacum shows more retention of homoeologs than Zea, indicating that the rate of fractionation varies between lineages. Substantial chromosomal rearrangements and downsizing appear to uniquely characterize Zea species. By using a wide sampling of genomes descending from a single WGD event, we gain a better understanding of the complex and dynamic processes of genome evolution after polyploidy and provide a framework for future exploration of other ancient polyploids.

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T20

ZmIRA1, a new root system architecture gene identified by phenomics

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Despite roots being a major organ essential to the well-being and survival of the plant the inherent difficulties in phenotyping them have resulted in inadequate knowledge of their genetic control. Recent techniques developed in our lab have allowed high-throughput quantification of roots in 2D and 3D to find genetic factors that regulate root system architecture (RSA). We applied these tools to the Illinois Long Term Protein Selection Strains (ILTPS) that have been recurrently selected for >100 years for their high and low protein content in the kernel, hypothesizing that the direct selection of nitrogen content in kernels also led to indirect selection of root phenotypes involved in nitrogen uptake. Using the recombinant inbred population of the ILTPS, the Illinois Protein Strain Recombinant Inbreds (IPSRIs), we performed a genome-wide association study (GWAS) with root traits extracted from 2D images of field-grown maize. A gene that we will refer to as Ideal Root Architecture 1 (ZmIRA1) was identified and is thought to function in central carbon-nitrogen metabolism. ZmIRA1 was confirmed to have promoter indels and differential expression between Illinois High Protein (IHP) and Illinois Low Protein (ILP) inbred lines, defining two alleles. In field experiments conducted in 2020 and 2022 with the IPSRI population, field-grown root crowns were measured from X-ray tomography-generated models and a custom feature extraction pipeline. We found that possessing either the IHP or ILP allele for ZmIRA1 was correlated with phenotypic differences in maize RSA. Subsequent examination of ZmIRA1 was done by CRISPR-Cas9 knockout mutants in a B73 background. In a greenhouse experiment with high nitrogen, we found that the mutants have specific root system architectural differences compared to the WT validating the potential link between central metabolism and root growth.

T21

Genetic and environmental impacts on the maize microbiome across the United States

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Microbes live on, around, and inside maize, yet we know relatively little about what most of them are doing and how they impact the plant. Two key questions are to what degree the local environment and host genetics shape these microbial communities, and what sort of interactions happen between them. To address these questions, we performed two analyses of maize microbiomes across the US as part of the Genomes to Fields Initiative. Our first analysis focused on soil and endophyte microbiomes from young (<1 month old) plants at 30 locations. We confirm that the plant acts as a strong filter, with endophyte communities severely restricted relative to soil and rhizosphere ones. We also find that endophyte communities are much more interconnected than soil and rhizosphere communities, with many more microbe-microbe interactions present. We find few consistent effects from specific environmental variables (pH, nutrient levels, etc.), and soil and rhizosphere communities are actually more consistent across locations than endophyte communities. Our second analysis focused on stalk endophytes in 20-30 hybrids at 20 locations, sampled at anthesis. We find that host genetics by itself has almost no consistent effect across environments, yet gene-by-environment interaction is substantial. This pattern holds across multiple measures of microbial diversity and is consistent with previous data from the maize rhizosphere. These results imply a 2-step model where the environment determines the initial diversity that is then modified by host genetics. This modification is not consistent across environments, implying there is no "ideal" microbiome for a genotype. We do find associations between the stalk endophyte community and environmental conditions (especially soil potassium), which merit further investigation. Taken together, these results indicate that the maize microbiome is a complex trait subject to strong gene-by-environment interaction, and this fact needs to be taken into account when designing future experiments.

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T22

Exploring the *cis*-regulatory landscape of the B73 and Mo17 genomes

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The domestication and improvement of many plant species frequently involved modulation of transcriptional outputs and continues to offer much promise for targeted trait engineering. The *cis*-regulatory regions controlling trait-associated transcriptional variants however reside within non-coding regions that are currently poorly annotated. This is particularly true in large crop genomes such as maize where regulatory regions constitute only a small fraction of the total genomic space. The current understanding of transcriptional regulation in eukaryotic organisms with large genomes posits that transcription factors (TFs) bind to short DNA sequence motifs (TF binding sites or TFBSs) in small regional clusters of accessible chromatin called cis-regulatory modules (CRMs), and control the gene expression changes responsible for plant developmental programs and environmental responses. CRMs can be in proximity of core promoters, upstream and downstream of the genes they regulate, and can even be located tens or hundreds of kilobases away from their regulated targets, complicating their identification. While sequences matching TFBS motifs are highly abundant within a genome, only a very small fraction is actually bound by TFs and affect gene expression. To improve the characterization of the functional regulatory space in maize, we used DAP-seq (DNA-affinity purification sequencing) to profile binding sites for ~200 TFs belonging to 30 distinct TF families in both B73 and Mo17 genomes. This allowed us to capture the DNA binding landscape of distinct TF families, and to assemble a panel of 66 TFs that represent a large portion of TFBS diversity. Overlap with orthogonal datasets of accessible chromatin regions uncovered the portion of the genome occupied by functional CRMs. Comparative analysis of TF binding in the two genomes revealed extensive variation in position, organization and structures of CRMs; the majority of this diversity is caused by existing structural variation. Guided by high-resolution maps of proximal and distal CRMs, we are disrupting specific CRMs and measuring phenotypic outcomes, with a focus on genes regulating plant architecture and biotic stresses. Our approach identifies functional CRMs and ultimately aids in understanding how TF-DNA binding and its variability in different genetic backgrounds affects gene regulation and ultimately phenotypic outcomes in maize.

Funding acknowledgement: National Science Foundation (NSF)

T23 @sohyun_bang

Understanding the role of *ZmWUS1* and *cis*-regulatory elements in maize inflorescence development at single-cell resolution

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Maize inflorescences arise through cell divisions from a group of undifferentiated stem cells within the inflorescence meristem. The organization of stem cells in the meristems is maintained by *WUSCHEL (WUS)* in the organizing center, which is situated beneath the stem cells. *WUS* encodes a homeodomain transcription factor whose expression is regulated by a negative feedback loop with *CLAVATA3*. The *Barren inflorescence3* (*Bif3)* mutant in maize is caused by a tandem duplicated copy of *ZmWUS1*, and it displays phenotypic alterations in ear, enlarged inflorescences and reduced numbers of spikelet-pair meristems. We hypothesized that *ZmWUS1* overexpression alters gene expression by modifying *cis*-regulatory elements (CREs) near target genes. The precise target region of *WUS* remains elusive due to restricted expression and activity of *WUS* in specific cells. Here, we performed an *in vivo* approach, single-cell Assay for Transposase-Accessible Chromatin with sequencing (scATAC-seq) in early developing ears in wild type and *Bif3* mutants to identify CREs affected by overexpressed *ZmWUS1*. We identified 14 distinct cell types, including organizing center with high chromatin accessibility around *ZmWUS1* correlating with its known expression. Using the *ZmWUS1*-bound motif TGAATGAA, obtained in DNA Affinity Purification and sequencing (DAP-seq) experiments, we found *ZmWUS1*binding activity in outer cells, encompassing stem cells. This suggests *ZmWUS1* migration from the organizing center to outer cells. However, alternative CREs were observed in the organizing center of *Bif3*, but not in outer cells. This indicates that in *Bif3*, excess *ZmWUS1* binds non-target CREs in the organizing center, leading to altered gene regulation. Further investigation revealed increased chromatin accessibility in CREs of the *Bif3* organizing center, enriched with the motif TWAT, distinct from the one identified in DAP-seq. These findings show that altered *ZmWUS1* levels influence its binding behavior in the organizing center of *Bif3*, and may explain the drastic phenotypic changes observed in mutant ears.

Gene / Gene Models described: *ZmWUS1*; Zm00001eb067310
Funding acknowledgement: National Science Foundation (NSF)

T24 @Sunil_KumarKR

Integrating single-cell transcriptomes links organ-specific gene expression with function

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Root architecture determines spatiotemporal control of resource exploration and acquisition, leading to crucial implications in plant productivity. Little is known about cell type differences among these roots at the molecular level and how these affect cellular organization, development, and function. Here, we present a comparative study of single-cell transcriptomes from the embryonic primary and seminal roots and post-embryonic lateral and crown roots in maize B73. Integration of scRNAseq datasets combining more than 24,000 cells revealed conserved and unique transcriptional programs in cell type specification and allowed dissection of gene expression pattern changes within cell types. While primary and secondary roots morphologically differ in their number of cortical layers, we didn't see significant differences in the number of cells with cortical identity. However, a significantly higher number of cells showed endodermis identity in lateral roots, revealing a potential root-type specific transcriptional specialization. Post-embryonic crown roots showed an overrepresentation of genes involved in water transport, epidermis differentiation, and response to stress compared to primary and seminal roots. We also identified genes that switched cell-type-specific expression patterns across root types. For example, grassy tillers1, a class I HD-Zip gene that controls lateral branching, showed broad expression across all cell types in the lateral roots, while its expression was restricted to specific cell types such as pericycle and less differentiated xylem and phloem in primary and seminal roots, and almost absent in crown roots. The gt1 mutant phenotype consistently shows longer lateral roots, suggesting that *gt1*'s growth suppression phenotypes are conserved in shoot and root. These results together show the potential of scRNAseq in unraveling functionally relevant molecular complexity of root architecture in maize.

Gene / Gene Models described: *gt1*; Zm00001eb007950
Funding acknowledgement: National Science Foundation (NSF)

T25 @PlantEvol_Lee

Meiotic recombination differs between sexes in maize.

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Meiotic recombination is a fundamental biological process that exchanges genetic material between homologous chromosomes during meiosis. It involves the physical swapping of DNA strands, known as crossover (CO), which influences genetic diversity in offspring. However, a comprehensive understanding of the patterns and regulation of COs, especially in female and male meiocytes in maize, 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. We performed genotyping-by-sequencing (GBS) on approximately 3,000 backcrossed (BC1) individuals derived from 15 male and female F1 hybrids. Some BC1 hybrids showed significantly more COs in either males or females, particularly with higher recombination rates at the chromosome ends. In other lines, no significant differences were observed between sexes in COs at the whole-genome level, although some distinctions were found at the chromosome level. These results suggest that meiotic recombination differs considerably among different maize genetic backgrounds. 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.

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T26

x1 encodes a putative RNA-binding protein required for paramutations at multiple loci

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Paramutations are defined by *trans*-homolog interactions resulting in meiotically-heritable epigenetic changes affecting gene regulation. While the nature of these interactions remains unclear, an *Arabidopsis thaliana* (*A.t.*)-based 24 nucleotide (nt) RNA-directed DNA methylation (RdDM)-like mechanism provides one working model. In maize, paramutations occur between specific alleles at several loci including *purple plant1* (*pl1*) and *booster1* (*b1*) which encode transcription factors promoting purple/red pigmentation. Relative to highly-expressed reference states (*Pl-Rh* and *B-I*), paramutant derivatives (*Pl'* and *B'*) confer weak plant color and are exclusively transmitted from respective *Pl-Rh / Pl'* and *B-I / B'* heterozygotes in apparent violation of Mendelian segregation. In forward genetic screens for factors *required* to *maintain repression* (*rmr*) of *Pl'* states, ethyl methanesulfonate-induced mutations define at least sixteen loci. All seven known RMR proteins facilitate RNA polymerase IV-derived 24nt RNA biogenesis, yet no RdDM-like 24nt RNA effector proteins have been identified. Here we show the *rmr17* locus is genetically required for paramutations at both *pl1* and *b1*. Bulk-segregant WGS analysis and deletion-based validation map *rmr17* to the *x1* gene located within the highly-studied *a1–sh2* interval. *x1* encodes the founding member of a protein family with namesake XS domains responsible for binding double-stranded (ds) RNA. The likely *A.t.* X1 orthologs represent a clade of three non-redundant paralogs forming heterotetrameric complexes required for RdDM. These complexes 1) bind duplexes of 24nt RNA and RNA polymerase V-derived long non-coding RNA (lncRNA) scaffolds, 2) interact with the SWI3B nucleosome remodeler, and 3) help recruit the *de novo* cytosine methyltransferase DRM2. Our findings support a role for X1 in paramutation by recognizing 24nt RNA-lncRNA duplexes and recruiting proteins that facilitate meiotically-heritable repressive chromatin modifications. The identification and characterization of *rmr17* thus represents a significant advance in understanding the role of 24nt RNAs in defining heritable epigenetic variations.

Gene / Gene Models described: *x1*; Zm00001d044127

Funding acknowledgement: National Science Foundation (NSF), United States Department of Agriculture (USDA), The Ohio State University Center for RNA Biology (CRB)

T27

Strigolactone control of iron homeostasis in maize.

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Strigolactones are plant hormones with roles in a wide range of signaling and developmental processes. A yellow-striped maize mutant, (*interveinal yellow*) *ivy*, was determined to have low iron in tissues under normal growth conditions. The gene underlying the *ivy* mutation was mapped and identified as *ZmCCD8*, a key enzyme in the biosynthesis of strigolactones. Under iron-replete conditions, comparison of the transcriptomes of wild-type plants and maize *ccd8* mutants revealed suppression of several iron-regulated genes in *ccd8*. These genes are normally up-regulated during iron deficiency and include the key iron-regulated transcription factor *IRO2* as well as genes involved in the biosynthesis of iron chelators and transporters. Additionally, external supply of synthetic strigolactone in the mutants alleviated chlorosis and returned iron-regulated gene expression to wild-type levels. In iron limited conditions, iron-regulated gene expression in *ccd8* mutants responded normally, indicating that strigolactones are not required for the iron deficiency response. However, they are required for basal expression of iron-regulated genes when adequate iron is available, highlighting a distinction between iron homeostasis during normal growth, and the iron deficiency response. The connection between strigolactones and iron homeostasis is not limited to maize, as Arabidopsis *ccd8* mutants also show strong chlorosis when grown on medium with moderate levels of iron. This previously unappreciated role may have implications for the use of strigolactones in agricultural contexts.

Gene / Gene Models described: *ccd8*; GRMZM2G446858

Funding acknowledgement: United States Department of Agriculture (USDA)

T28

Upper level and cross hierarchical regulation of predominantly expressed phenolic genes in maize

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There is strong interest in deciphering the gene regulatory networks (GRNs) that govern plant specialized metabolism to assist in plant breeding. Here, we investigated the GRN governing phenolic biosynthesis pathways from which ~ 8000 plant specialized metabolites are derived. Previously it was established that 19 predominantly expressed maize phenolic (PEP) genes are sufficient to explain >70% of the metabolic flux through the phenylpropanoid, monolignol, and flavonoid branches of this pathway. A yeast-1-hybrid (Y1H) gene centric screening approach was employed to discover upper level (tiers 2, 3, and 4) regulators of maize PEP genes. These regulators were examined by co-expression analyses, and a subset of protein-DNA interactions (PDIs) validated *in vivo1* by ChIP-qPCR and luciferase reporter assays in maize protoplasts. We describe a comprehensive GRN composed of 429 PDIs that exhibits hubs with high connectivity and cross hierarchical regulation of PEP genes in different branches of the pathway. The core PEP GRN is composed of 21,16, and 1 transcription factors (TFs) that operate as tier 2, 3, and 4 regulators respectively. Of these, ZmC3H42 regulates tier 1, 2 and 3 genes and also exhibits autoregulation. The core GRN includes TFs and TFs that are conserved in other plant species and that are implicated in phenolic gene regulation (*e.g.,* ZmMYB40/53/100, ZmMADS9, and ZmWD40.1/PAC1). The GRN also includes conserved TFs (*e.g.,* ZmC3H9, ZmHB20/79, ZmNAC103/123, ZmMYB19/26, ZmMYBR87, ZmDOF3, ZmbZIP67, ZmTCP30, and ZmbHLH128) which indicate that maize PEP genes are under developmental regulation and also fall under the control of biotic and abiotic stress. Together, the PEP GRN describes a complex regulatory mechanism that has evolved to coordinately regulate many phenolic genes in response to multiple internal and external signals and can guide efforts aimed at manipulating phenolic levels in plants.

Funding acknowledgement: National Science Foundation (NSF, IOS-1733633)

T29

Elucidating the metabolomic and single-cell transcriptomic landscape of maize leaf senescence

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Delayed leaf senescence (a.k.a. staygreen) is associated with higher grain yield, increased total dry matter, and heat and drought tolerance. To resolve the genetic architecture of senescence, we generated a high-resolution map of physiological, transcriptomic, and metabolic processes underlying natural diversity for staygreen. Analysis of distinct physiological indicators of senescence revealed that there are multiple paths to staygreen traits specified by temporally and spatially distinct cellular processes. Time-course analysis of primary and secondary leaf metabolome in 10 inbred lines, five staygreen and five non-staygreen, at three developmental stages revealed substantial metabolic variation for staygreen in maize. Combinatorial analysis of metabolic and phenotypic variation using marginal scan revealed striking differences in metabolome and found that 44 (92%) primary metabolites and 1201 (96%) secondary mass features underlie variation for the staygreen trait. In-depth analysis of flux in the secondary metabolism revealed that flavonoids (naringenin chalcone, kaempferol, and eriodictyol) are crucial for discerning the staygreen phenotype. Functional analysis of these metabolites in Arabidopsis validated the role of naringenin chalcone and eriodictyol in delaying senescence. To enhance the resolution of transcriptional changes during senescence, we compared the single-cell transcriptome of a staygreen and a non-staygreen inbred lines and identified that bundle sheath or mesophyll cells regulate transcriptional activity. Remarkably, we identified 2 and 7 distinct clusters of bundle sheath and mesophyll cells, respectively, indicating transcriptional divergence within these cell types during senescence. Our findings will identify novel components of biological organization that regulate staygreen in maize.

Funding acknowledgement: National Science Foundation (NSF)

T30

Potent pollen gene regulation by DNA glycosylases in maize

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While DNA methylation primarily represses transposable elements (TEs), some genes are repressed by DNA methylation in plant body tissues and require demethylation by DNA glycosylases (DNGs) for activation in endosperm and pollen. Activity of either one of two DNGs, MDR1 or DNG102, is essential for pollen viability in maize. Using single-pollen mRNA sequencing on pollen segregating mutations in both genes, we identified approximately 60 candidate DNG target genes. While they are silent or barely detectable in the plant body, this small set of genes accounts for over 10% of the pollen polyadenylated transcriptome. They are unusual in their tendency to lack introns but even more so in their having TE-like methylation in their coding DNA sequence. They are strongly enriched for genes that are predicted to function in cell wall loosening to support the rapid tip growth characteristic of pollen tubes as they carry the sperm cells through maternal apoplast and extracellular matrix of the pistil. These results suggest a function for DNA methylation and demethylation in regulating genes with potential for extremely high expression in pollen but constitutive silencing elsewhere.

Funding acknowledgement: National Science Foundation (NSF)

Posters

P1

Annotation of gene-metabolite functional relationships using multi-omics mapping of the Sorghum Bioenergy Association Panel

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Plants synthesize a multitude of diverse metabolites crucial for plant survival and adaptation with implications in various biological processes and applications in agriculture and human health. However, the identity of most plant metabolites and the genes involved in their synthesis and regulation remain unknown. Thus, improving our understanding of plant gene-metabolite functional relationships is needed to unlock higher plant productivity and stress tolerance. We used a multi-omics approach combining untargeted liquid chromatography-mass spectrometry (LC-MS), LC-UV absorbance, and genome-wide association (GWA) to identify and characterize mass features and the alleles in genes responsible with their variation in the Sorghum Bioenergy Association Panel (BAP). Defense compounds, including salicylic acid (SA) and the dihydroxybenzoic acids (DHBAs), were identified. SA, 2,3 DHBA, and 2,5 DHBA levels are all variable dependent on genotype in the population. Additionally, several other compounds were identified including multiple flavonoids and dhurrin. Functional validation of both compound identities and gene functions were further explored via reverse genetics studies using a sequence-indexed EMS mutant population sorghum. The same untargeted LC-MS approach was used to profile a set of EMS mutants. One such mutant had a high-impact point mutation in CHALCONE SYNTHASE an important enzyme in flavonoid biosynthesis allowing the identification of a suite of flavonoids. Cross validation between the EMS mutant population and association mapping allows efficient validation, or rejection, of candidate genes identified by association mapping. Comparisons between LC-MS and LC-UV association data also help validate and identify mass features of interest. Additionally, total absorbance at specific wavelengths or the ratio of two specific wavelengths were mapped to identify alleles in genes associated with absorbance variations and higher abundances of light-absorbing compounds. LC-UV total absorbance association mapping can be compared and applied to field hyperspectral data, thus providing another application for the wealth of hyperspectral data collected from field studies.

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

P2

Autoimmune mutants reveal new immunity pathways in maize

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The plant immune system includes constitutive and induced responses, when the strict fine-tuning regulation is disrupted autoimmunity may occur, i.e. spontaneous initiation of plant defense response affecting plant performance. In a screening to look for developmental affected maize mutants we identified *narrow odd dwarf* (*nod*) and *Liguleless narrow* (*Lgn*); surprisingly, transcriptome analysis showed overrepresentation of defense related gene categories, suggesting autoimmunity in these mutants. Proteomic analysis of NOD and LGN immunoprecipitated complexes showed interaction with exocyst components and MAPKs, and NOD with LGN interaction, BiFC experiments showed plasma membrane localization of NOD, LGN and some interactors relating these proteins with MAPK signaling pathways. NOD is the maize ortholog of the Arabidopsis MCAs (*Mid-Complementing Activity 1* and *2*), which have been reported forming a calcium import channel, based on the 3D structure and the MLKL domain, recently MCA2 was proposed to be a hNLR protein. In addition, NOD was identified interacting with *P. maydis* effectors in a Y2H screen, suggesting that NOD may be an atypical NLR. To test whether NOD can produce cell death, we did transient expression in *N. benthamiana*, showing that NOD N-terminus domain is enough to produce cell death. To explore the *nod* and *Lgn* involvement in pathogen response we are using *Setosphaeria turcica* to infect mutants in different genetic backgrounds. We found differential expression patterns of pathogenesis-related genes in B73 and Mo17, suggesting that those proteins are preventing PR genes induction depending on genetic background. Next experiments with fungal effectors will give information about NOD and LGN function at molecular level during pathogen infection in maize.

Gene / Gene Models described: *nod, Lgn1*; Zm00001eb004320, Zm00001eb382080
Funding acknowledgement: National Science Foundation (NSF), UC Mexus-CONACYT

P3 @julianayassitepe1

Challenges and insights from establishing a maize transformation laboratory

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Maize genetic transformation, especially that mediated by Agrobacterium tumefaciens, is a crucial method for creating genetically modified varieties. Besides its extensive utilization for generating transgenic events, it is currently the method of choice to deliver CRISPR/Cas9 machinery into maize cells, allowing the production of genome-edited genotypes. In recent years, significant advancements in maize transformation protocols have been made. These developments have gained attention from both academic and industry organizations, resulting in the emergence of several public and private maize transformation laboratories and facilities worldwide. In 2019, we established maize transformation capabilities at the Genomics for Climate Change Research Center (GCCRC) in Campinas, Brazil, as part of a plant biotechnology pipeline using maize immature zygotic embryos of the model temperate inbred line B104. We observed a significant increase in transformation efficiency after incorporating the utilization of ternary vectors and morphogenic regulator genes (MR). The “altruist” method increases transformation efficiency and the number of fertile plants by avoiding the adverse effects caused by defective MR excision when using a single vector for the target and MR genes. With these optimizations, we can now transform and edit tropical maize lines more efficiently than B104. Seasonal variation has a significant impact on embryo production, which is highly correlated with transformation efficiency. Currently, we are testing a leaf-based protocol, which has shown promising results in overcoming this issue.

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P4 @NadiaNazihM

Changes in sorbitol metabolism impact kernel sink strength and seed size

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Maize endosperm development is characterized by an oxygen-deficient microenvironment, requiring molecular and biochemical mechanisms for hypoxia acclimation and maintenance. Sorbitol dehydrogenase (SDH) catalyzes the conversion of (fructose + NADH)↔(sorbitol + NAD^+) in endosperms during grain-fill. We hypothesize that SDH aids endosperm development in at least two ways, 1) by regenerating the NAD^+ that maintains redox balance and glycolytic flux in the hypoxic endosperm, and 2) by enhancing sink strength through the metabolism of fructose and indirectly the import of its precursor sucrose. In support of our hypothesis, sorbitol accumulates in the endosperm region with lowest oxygen levels as determined by FTIR imaging in developing wild-type kernels. To evaluate the role of SDH, we characterized an *Ac/Ds*-induced *sdh1* mutant which lacks detectable SDH activity and accumulates little to no sorbitol in kernels. We found that *sdh1* mutant ears bear smaller kernels beginning at 15 DAP, with 13-17% less dry weight at maturity, suggesting an important role in kernel filling. Metabolic analysis of *sdh1* mutant kernels shows elevated levels of fructose as well as sucrose (100% and 25% greater, respectively). Results indicate that conversion of fructose to sorbitol via SDH promotes sucrose metabolism and import. Work in progress focuses on characterization of *Sdh1* over-expression lines, development of double-mutant kernels deficient in both sorbitol and starch biosynthesis, and an in-depth evaluation of metabolic impacts. Findings thus far highlight the role of SDH in kernel development with implications for metabolic engineering strategies to enhance sink strength and composition of the maize grain.

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

P5

Characterization of the maize membrane lipidome in a large collection of introgression lines derived from crosses between B73 and traditional maize varieties and teosintes

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After domestication, maize was transported to the highlands of Mexico, where it crossed with a highland teosinte, Zea mays ssp. mexicana. This genomic introgression persists in modern maize inbreds and carries genes important for abiotic stress tolerance, particularly those involved in highland adaptation. Stresses typical of highland environments include high UV radiation, low temperature, and low phosphorus availability. Low-temperature stress, in particular, impacts the ability of tropical plants like maize to survive in colder environments. One way that plants adapt to low-temperature stress is through alterations in membrane lipids, which in turn impact membrane fluidity and structure. Using a biparental B73 x Palomero Toluqueño (a highland traditional maize variety from central Mexico) RIL population, we previously identified a gene, High Phosphatidylcholine 1 (hpc1), whose impaired function in highland maize impacts the amount of phosphatidylcholine, a key membrane lipid, and aids in highland adaptation. Here, we seek to quantify the extent of variation in membrane lipids and map the underlying genetic variation using a newly developed collection of introgression lines derived from crosses between B73 and hundreds of accessions of traditional maize varieties and teosintes from all across Latin America.

Gene / Gene Models described: *hpc1*; Zm00001eb121780
Funding acknowledgement: United States Department of Agriculture (USDA)

P6

Characterizing expression of meristem genes in a Lig5 maize mutant

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Plant meristems are developmental tissues that shape the structure of all plant organs. Aberrations in expression of meristematic genes as well as mutations in the proteins active in meristematic tissues could affect plant development. Understanding the relationships between meristem-specific genes and unveiling the regulatory mechanisms for their expression is essential to controlling plant development and breeding crops with desired characteristics. Liguleless genes are a group of meristem-specific genes affecting plant leaf development. A Liguleless5 (Lig5) mutation causes aberrations in the leaf ligule, a thin outgrowth at the junction of leaf and stem, changing the shape and the angle of seedling leaves. In addition, this mutation causes knot formation on leaves. In this project, we investigated the expression patterns of meristem-specific genes from several families (knotted, knotted-like, and liguleless) in meristematic and leaf tissues in lig5 mutant in comparison to the wildtype, to understand the possible causes of this phenotype. RNA was extracted from the meristematic (stem) and leaf tissues in young seedlings, converted into cDNA, and analyzed using qPCR to determine gene expression. We determined that several meristem-specific genes were overexpressed in the mutant leaf tissues and underexpressed in the mutant meristematic tissues compared to the wild-type plants. A few of those genes that expressed a change in expression patterns were kn1, knox1, knox2, and knox3. Our findings suggest that lig5 mutation causes changes in expression patterns of meristem-specific genes. Further studies are needed to understand the details of this effect.

Funding acknowledgement: National Science Foundation (NSF)

P7

Comparative study of approaches for gene activation in maize

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Modulating gene expression is an essential tool for understanding biological processes and advancing the field of synthetic biology. This study investigates the efficacy of three methods in maize for stably activating gene transcription. The first method involves the designer Transcription Activator-Like effector (dTALe), a programmable protein functioning in specific DNA binding and gene activation. The second method uses the dead Cas9 (dCas9) activation, an alternative programable system for transcriptional modulation. The third one employs standard overexpression of a transgene of interest with a strong promoter. To assess the magnitude of transcriptional activation of targeted genes, we are developing transient expression of each approach. The goal of this project is to provide information to identify optimal strategies for achieving targeted gene activation in maize. Our poster will discuss what we have developed and the issues we encountered.

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

P8 @shivreet_kaur

Delving into the biochemical underpinnings of disease resistance for southern leaf blight in maize

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Southern leaf blight (SLB), a foliar disease of maize caused by the fungus *Cochliobolus heterostrophus* poses a significant threat to maize production globally. Tapping into the host genetic resistance proves to be a potent approach for managing SLB. There have been considerable efforts made towards identifying and elucidating the genes involved in SLB resistance, however, the mechanisms by which these genes operate are often uncertain. The intricate biochemical changes that transpire during plant-pathogen interactions bear significant biological relevance, making metabolomics an ideal tool for in-depth exploration. In this study we are using a set of 10 near isogenic lines (NIL's) in the genetic background of the B73 inbred line, which are significantly more or less susceptible to SLB than B73 (2 more susceptible, 8 more resistant). The lines were phenotypically screened for SLB in 2019 and 2021 in both infected and uninfected conditions across the growing season. Inoculated and uninoculated samples collected at 2 time points post inoculation from both the seasons were subjected to an untargeted LC-MS/MS. Preliminary principal component analysis indicated a consistent clustering of the biological replicates by genotype, treatment and timepoint, affirming the robustness of the data. Upon SLB infection, we saw a significant upregulation of various amino acids in addition to several metabolites involved in defense related pathways such as the phenylpropanoid pathway in the resistant lines compared to B73. We will further investigate the corresponding uninoculated comparisons and see if such differences are conserved. Through this approach, our aim is to pinpoint metabolites involved in increased SLB resistance. Given our solid knowledge of the genetic framework for resistance in these lines, by combining our metabolomic quantitative trait locus (mQTL) data for the identified metabolites with SLB resistance QTLs, we aim to identify the genetic and biochemical factors contributing to disease resistance.

Funding acknowledgement: United States Department of Agriculture (USDA)

P9

Disruption of allene oxide cyclase results in jasmonic acid deficiency despite incomplete loss of 12-OPDA

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Allene oxide cyclases (AOC) convert allene oxide to 12-oxo-phytodienoic acid (12-OPDA), a required step in jasmonic acid (JA) biosynthesis. 12-OPDA and JA represent important signaling molecules that modulate responses to biotic and abiotic stresses while also regulating developmental processes. They are part of a larger group of bioactive oxylipins that can be found in free and lipid-bound forms. We used gene editing to disrupt both copies of AOC in maize. Unexpectedly, AOC double mutants retained the capacity to produce 12-OPDA, accumulating approximately 20% of normal levels. Despite this, double mutants failed to accumulate appreciable levels of jasmonates downstream of 12-OPDA, including jasmonic acid (JA) and JA-isoleucine. Double mutant plants displayed the expected JA deficient phenotypes, including feminization of male florets and extreme susceptibility to insect and necrotrophic fungal pathogens. We have conducted extensive metabolic and gene expression analyses on these mutants, providing a detailed view of the effects of losing JA on defense responses. Strikingly, free oxylipins are severely reduced in double mutants while lipid-bound forms accumulate in response to herbivory. We provide speculation regarding the source of the non-AOC derived 12-OPDA, reasons why the 12-OPDA made by double mutants fails to translate into JA production, and possible explanations for the inversion of the bound vs unbound oxylipin accumulation responses in AOC mutants compared to normal plants.

Gene / Gene Models described: *aoc1, aoc2*; GRMZM2G415793, GRMZM2G077316
Funding acknowledgement: United States Department of Agriculture (USDA)

P10

Elucidating freezing tolerance in tripsacum: Insights from combined transcriptome and proteome

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By extending the growing season of annual crops, we can improve net primary productivity and yield, and address the mismatch between soil nitrate availability and crop nitrate demand. In temperate regions, early maize planting requires cold tolerance, a trait not present in current elite maize varieties. Tripsacum dactyloides, the closest perennial and cold-tolerant relative of maize, offers a potential source for identifying genes related to cold adaptation. To explore this, we crossed Tripsacum accessions collected from both high- and low-latitude habitats. The resulting hybrids were screened in a field trial in Ithaca, NY, over two years, a location known for its deep freezing winter temperatures. We analyzed protein expression levels in the hybrids' rhizomes and roots, taken during winter (dormant stage) and summer (active stage), using a shotgun proteomics approach. This analysis was combined with RNAseq data from leaf tissues of freezing tolerant seedlings before and after cold acclimation treatments, sampled from F2 families derived from the aforementioned hybrids. Our examination of significant genes, overlapping between the seedling transcriptomic and rhizome proteomics data, identified 109 up-regulated and 161 down-regulated proteins common to all three data sets. We compiled a list of cryoprotectants, including those previously known to be involved in abiotic stress, such as late embryogenesis abundant (LEA) proteins, heat shock proteins, and ABA stress ripening genes. Our results present variations in copy number, protein structure, and transcription factor binding sites in comparison to orthologs in maize.

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P11

Empirical assessment of the Wisconsin Crop Innovation Center high throughput maize gene editing pipeline

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Gene editing holds great promise as a significant advance for modern crop plant breeding. To facilitate large scale *Agrobacterium* transformation mediated gene editing projects in the elite inbred maize cultivar LH244, the Wisconsin Crop Innovation Center combined the recently published *WOX2A* with the BLACKSMITH polycistronic guide RNA expression cassette assembly system to produce MAIZEEDIT binary plasmids. These constructs hold up to four gRNA and feature the Seed RUBY marker gene for non-destructive identification of transgenic and null segregant kernels. In this experiment, nine MAIZEEDIT plasmids were designed, assembled, and transformation and gene editing performance was assessed, for targeting with two guides per gene a total of 17 genes. Molecular and visual analyses were combined to evaluate 162 plants generated from the nine constructs, 130 of which were found to be transgenic via end-point PCR detection of the Seed RUBY marker gene. An amplicon sequencing approach was used to assess gene editing in T_0 plants, and gene editing metrics, as well as Seed RUBY performance, are reported. One construct contained gRNA targeting the *phytoene synthase* gene (*y1*; also called *yellow endosperm 1*). Interestingly, some ears which displayed *y1* editing, based on visual kernel phenotype, occurred on ears on which Seed RUBY phenotype was not observed. While numerous examples of the expected 3:1 segregation ratio of RUBY:non-RUBY endosperm were observed, consistent with single-locus transgene insertion, multiple instances of unexpected segregation ratios were also found. The frequency of phenotypes observed on ears in which kernel traits were targeted supported that editing occurred early in the transformation process resulting in ears and tassels that were uniformly transmitting edits. Molecular and phenotype analyses of T_1 progeny will confirm the operational functionality of the WCIC MAIZEEDIT pipeline to produce, via *WOX2A*-based transformation combined with polycistronic tRNA delimited gRNA, stable and heritable edited loci.

Funding acknowledgement: United States Department of Agriculture (USDA)

P12

Engineering a terpene-depleted chassis in tomato fruit for production of high value terpenes

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Plants produce numerous specialized metabolites that function in plant defense, as attractants to pollinators and symbionts, and in cell wall strengthening. Over 50,000 plant terpenoids are known which are synthesized from universal C_5 building blocks via the activity of terpene synthases, cytochrome P450s, and glycosyl transferases. A subset of these specialized metabolites are highly valued for a range of properties including insect repellents, fragrances, antimicrobial compounds, low calorie sweeteners, and medicinal properties. It is challenging to economically or sustainably produce many of these compounds from their native species due to the low abundance of the compounds or the time and space required to grow sufficient plants for production of the natural product. In addition, heterologous expression of terpenoid biosynthetic genes in plants is problematic due to the tendency for conversion of the activated products into conjugates or other derivatives via endogenous cytochrome P450s, and glycosyl transferases. In this project, we are using gene editing and transformation technologies to overcome these challenges by creating a novel tomato chassis with minimal fruit terpenoid biosynthetic capacity through removal of native terpenoid biosynthetic pathways, that will enable engineering of high value terpenoid molecules in tomato fruit.

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P13

Engineering root N remobilization after grain filling to decrease greenhouse gas emissions and N leaching in corn agriculture

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The extensive use of nitrogen (N) in maize agriculture is a major contributor to environmental challenges, including waterway N pollution and N_2O emissions. Enhancing maize N use-efficiency and recycling can contribute to mitigate these environmental impacts. Perennial grasses, including maize relatives of the Andropogoneae tribe, have evolved efficient N recycling strategies, making them a valuable genetic reservoir to improve N management in maize. One of these N-recycling strategies is the remobilization of N from aboveground to underground tissues that perennial grasses activate post-flowering and during the winter. This study aims to engineer perennial-like root N remobilization in maize after grain filling to reduce N leaching and N_2O emissions. Our approach involves identifying key genes for perennial N-remobilization through seasonal tracking of N, amino acid and gene expression profiles in both roots and leaves of multiple Andropogoneae species grown under field conditions. We will also conduct a detailed analysis of gene expression and total N profiles in B73 maize with *Zea diploperennis* introgressions. This analysis will span from the flowering stage to senescence, under field conditions. Additionally, we will compare these profiles in plants with and without a developing ear (sink) to pinpoint key genes responsible for root N remobilization in the absence of an N sink after flowering. Following the identification of key genes, we will employ comparative evolutionary genomics within the Andropogoneae tribe to further refine our selection of candidate genes for root N remobilization. These genes will subsequently be introduced into maize using genetic engineering or introgression techniques. This study aims to contribute to reducing greenhouse gas emissions and N leaching, paving the way for more sustainable maize agriculture practices.

Funding acknowledgement: United States Department of Agriculture (USDA)

P14

Exploring the genetics of drought resilience in sorghum

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Drought stress, a major threat to global food security, is intensifying due to climate change. Among cereal crops, sorghum stands out for its remarkable drought resilience, demonstrating superior adaptability to water scarcity compared to other major cereals. Under drought stress, sorghum exhibits a multifaceted response. Leaf rolling and stomata closure reduce transpiration, while photosynthetic efficiency adapts for optimal water use. Reduced growth prioritizes resources, and root architecture adjusts for enhanced water uptake, ensuring survival in harsh conditions. Here, we explore the genetic foundations of sorghum drought resilience through whole-plant phenotyping of a sequence-indexed, EMS mutagenized sorghum population using an automated, high-throughput field scanner system in Maricopa, AZ. This allowed us to identify mutants displaying various responses to drought stress, including those with altered photosynthetic efficiencies and exaggerated leaf rolling. Among these, the *leaf rolling 1* (*lr1*) mutant exhibited a severe leaf rolling phenotype even in response to mild environmental stress. Characterization of this mutant also revealed a defective root system. Analysis of an F2 mapping population indicated that both traits were recessive. We performed bulked segregant analysis using DNA pools from individuals with either the leaf rolling or defective root phenotypes and showed that a single locus on chromosome 9 was responsible for both traits. Further characterization of the *lr1* mutant through 3D reconstruction of root development, controlled stress experiments, and gene editing will provide mechanistic insights into root-to-shoot responses to drought stress. Overall, our field-based analyses of mutant phenotypes allow us to link genotype to stress-responsive phenotype, unraveling the genetic intricacies of sorghum's drought resilience and paving the way for the development of drought-resistant varieties crucial for ensuring food security in a changing climate.

Funding acknowledgement: Department of Energy (DOE)

P15 @jrrygnzlz

Finding a high throughput DNA extraction method for maize

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With the world's climate becoming more extreme it has imposed great challenges on agriculture and food security. Maize is a staple crop that much of the world depends on and it is vital to ensure it can overcome these stressors and flourish under these harsher growing conditions. The main method in getting corn to adapt to these new stressors is to create better varieties with better attributes to overcome these stresses. A key component in making this possible is to make the highly genetically diverse tropical corn lines available to breeding programs and maize geneticists around the world and not have it limited to the tropics due to photoperiod sensitivity. The three main genes suspected to suppress flowering of tropical corn in long day (temperate) latitudes are ZmRAP2.7, ZmCCT9, and ZmCCT10. In our lab the process to screen for different combinations of functional or edited alleles of these genes is very slow and tedious. It requires many steps, physical labor, and processes a very low number of samples at a time. On average for one person to prepare DNA from 20 leaf samples with our current protocol takes 3 to 4 hours. My goal is to create a DNA extraction protocol for maize that is affordable, fast, high throughput, and produces high quality DNA ready for PCR so that the plants can be screened more quickly and This would help other labs working with maize or similar DNA because they would be able to extract DNA faster and more efficiently.

Gene / Gene Models described: *ZmCCT9, ZmCCT10, ZmRAP2.7*; GRMZM2G004483, GRMZM2G381691, GRMZM2G700665
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P16

Functional genomics of coordinated nitrogen and phosphorous signaling in maize

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Few agricultural soils provide sufficient nitrogen (N) or phosphorous (P) to maximize the yields of modern corn hybrids. Consequently, nearly 7 million tons of N and 2 million tons of P are applied to US soils annually for corn production, at a combined farm price in excess of $5 billion. Reducing fertilizer inputs offers immediate economic and environmental benefits and will contribute to more sustainable cropping systems. Despite recent progress from Arabidopsis and rice that increases our understanding of N and P signaling, detailed functional information on these processes in maize is lacking. Prior work from our groups using genetic mapping, plant physiology, and gene expression profiling approaches supports the hypothesis that interactions among specific members of the *NITRATE TRANSPORTER1.1* (*Nrt1.1*), *PHO1*, and *SPX* protein families mediate coordinated responses to fluctuations in N and P supply. A collection of variant alleles for these three gene families has been obtained from natural diversity, transposon mutagenesis, and targeted CRISPR/Cas9 editing. We share an update of our phenotypic analyses of single and combined genetic variants. Three important findings to date include: 1) mutations in different *NRT1.1* paralogs vary for their impact on N uptake and growth responses to N; 2) a *nrt1.1* mutant suppresses enhanced responses to P of a *pho1;2a* mutant; and 3) analyses of patterns of genetic diversity suggest variants with reduced sensitivity to N and P supply are associated with recent breeding improvements in nutrient utilization among U.S. Corn Belt maize hybrids.

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P17

GRASSIUS 2.0 Knowledgebase: Updated resources and tools for regulomics in the grasses

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Grass species, which include the major cereal crops maize, wheat, rice, and sugarcane, are an integral part of our global agriculture and source of food and energy for a growing world population. GRASSIUS was established as a knowledge base for transcription factors (TFs) and coregulators (CRs) in maize and several other species in the grass family. TFs are a primary component of the gene regulatory networks (GRNs) and the underlying gene regulatory grids (GRGs) that govern all aspects of plant growth and metabolism. GRASSIUS is the source for all information pertaining to the maize TFome collection, which serves as a powerful resource for the discovery of GRNs in corn and other cereals (Burdo *et al*., Plant J, 2014 80: 356-66, Yang *et al.,* 2017. Mol Plant, 10:498–515). Here we announce the release of the GRASSIUS 2.0 knowledgebase (www.grassius.org) with updated data, query, and tool features, as well as the ability to expand to accommodate future datasets. The membership and annotation of all TF and CR families has been updated and revised to include gene models from versions 3, 4, and 5, of the maize genome. A translation tool enables cross referencing of Gene IDs between versions of the maize genome. A new Protein-DNA Interaction (PDI) Collection has been added that incorporates PDIs derived from ChIP, ChIP-seq, DAP-seq, pChIP, and Y1H datasets. A filtering tools permits the selection of PDIs within a ± 2kb distance from the transcriptional start site of a gene of interest. A new BLAST tool facilitates searching of the maize TFome as well as v3, v4, and v5 TF gene model sets. Lastly, we describe the methodology used to implement GRASSIUS 2.0 which can guide others in developing and updating similar plant gene regulatory knowledgebases. This project was funded by NSF grant IOS-1733633.

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P18

Gene editing of two maize chalcone synthase paralogs results in conditional male sterility

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The efficient production of hybrid seed remains a key cost of goods challenge in corn, due to the need for sterilization of the male. For decades, manual and mechanical detasseling has been used to eliminate unwanted pollen in hybrid production fields, complemented by the more recent implementation of cytoplasmic male sterility (CMS), and other proprietary hybridization systems. Looking for novel gene targets to create genic male sterility, we returned to a pair of classic maize mutants *colorless2* (C2) and *whitepollen1* (WHP1). In the homozygous double mutant, mutations in these two genes are reported to result in a flavonol-complementable-male sterility. Within the maize flavonol biosynthetic pathway, we identified a similar pair of male-expressed genes, with predicted chalcone synthase activity, *chalcone synthase2* (CHLS2) and *chalcone synthase11* (CHLS11). We created null mutants for these two genes using CRISPR-Cas12 technology and confirmed editing with Taqman and sanger sequencing. Interestingly, and contrary to the stable male sterility observed in *c2 whp1* double mutants, these edited lines demonstrated a conditional male sterility phenotype in the homozygous double mutant.

P19 @AmanArora_7

Gene expression identifies a role of maize ortholog of dwarf and low tillering (*gras42*) in brassinosteroid signaling

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Maize above-ground architecture, primarily affected by plant height and leaf architecture, is regulated by phytohormones including brassinosteroids (BR), gibberellins (GA), and auxins. Architectural mutants with defects in the biosynthesis or signaling of these hormones identify components of these pathways and enhance our knowledge about plant growth and development. We characterized three mutant alleles of *gras42*, the maize ortholog of rice *DWARF AND LOW TILLERING* (*DLT*) gene. The *gras42* mutants have semi-dwarf stature, shorter and wider leaves, and more upright leaf angles, and resemble the expected phenotype for a weak loss of BR signaling. In rice, DLT is a positive regulator of BR signaling. In Arabidopsis, this gene plays a role in cell cycle-regulated gene expression. An RNA-seq analysis of the expression consequences in the maize *gras42-mu1021149* loss-of-function mutant matched a weak loss of BR signaling, consistent with its previously demonstrated role in BR signaling in rice. This was demonstrated using a parametric index calculated from the expression of experimentally determined BR-responsive genes. A coordinated increase in the transcript levels of BR-repressed genes and decrease in the transcripts of BR-induced genes was observed in the *gras42* loss-of-function mutant. The double mutant phenotypes of *gras42-mu1021149* with *na1-1* and *d1* also indicated that *gras42* encodes a positive regulator of BR-responsive gene expression. Like other BR deficiencies, *gras42-mu1021149* effects on tassel development were suppressed by loss of GA in the *d1* mutant and suppressed the promotion of tiller growth by loss of GA. In single-cell transcriptomic data, GRAS42 expression was found in cells in the G2/M phase of the cell cycle, including nascent stomatal complexes, suggesting a conserved role for *gras42* in cell cycle gene expression between maize and Arabidopsis. In addition, a GWAS of natural variation in GRAS42 expression identified known BR pathway genes as regulators of *gras42* expression.

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P20

Genetic architecture of stalk lodging resistance in maize

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Stalk lodging severely limits crop yield and causes global annual losses of at least 6 billion USD in Maize (*Zea mays* L.). The genetic architecture of stalk lodging resistance remains poorly resolved due to 1) a poor understanding of phenotypes contributing to stalk strength and 2) lack of standardized phenotyping approaches to record these intermediate phenotypes. We generated a high-resolution phenome of maize stalks on 40 geometric, structural, and biomechanical phenotypes in a diversity panel, examined the impact of these traits on stalk lodging resistance, and identified underlying loci. Preserving the field location and identity of individual plants revealed substantial intra-plot variation indicating the effect of micro-environment on the phenotypic continuum of the intermediate phenotypes. The single-plant data allowed us to account for spatial effects and increase the accuracy of phenotype data for genetic analyses. Genetic correlations revealed a stronger association of intermediate phenotypes measured on the lower-most elongated internode with stalk flexural stiffness as compared to those measured on the primary ear-bearing internode. Most of the phenotypes under study exhibited low to moderate heritability indicating low genetic tractability and complex genetic inheritance. Multi-trait genome wide association analyses of combinations of different biomechanical, structural, and geometric phenotypes showed candidate genes involved in plant and ear height development, electron transport, membrane structure and transport, oxidoreductase activity, transcription factors, etc. Interestingly, majority of the SNPs identified are associated with the non-coding regions of the genome and certain SNPs are shared between different trait combinations indicating pleiotropic regulation of stalk lodging resistance. Characterizing novel candidate genes and regulatory variation identified in the present study would open avenues for disentangling the genetic architecture of stalk lodging resistance in maize.

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P21

Genetic interaction analysis of maize meristem regulators

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The formation of the plant body proceeds post-embryonically through the coordinated action of small groups of stem cells called meristems. Maintenance of meristems is mediated by the *CLAVATA-WUSCHEL* negative feedback loop. Aberrations in this well-conserved pathway result in either enlarged or prematurely terminated meristems. As such, in maize, grain yield is ultimately determined by the size of the female inflorescence meristem (IM) which dictates the number of rows of kernels in an ear of corn. Prior characterization of *ramosa1 enhancer locus2 (rel2)* mutants revealed pleiotropic vegetative and reproductive phenotypes such as defective axillary meristem initiation and IM maintenance in single recessive mutants. The REL2 family of transcriptional corepressor proteins is composed of four members, *REL2, REL2-LIKE1 (RELK1), RELK2* and *RELK3*. Interestingly, *rel2;relk1* mutants display enhanced enlargement in female IMs. However, it is currently unclear whether *REL2-RELK1* function in the canonical *CLAVATA-WUSCHEL* pathway or in parallel pathways. To distinguish these possibilities, we performed a genetic interaction analysis with *rel2* and known meristem maintenance mutants: *thick tassel dwaf1 (td1), fasciated ear3 (fea3)*, and *fasciated ear4 (fea4)*. *TD1* encodes an LRR-receptor-like kinase, *FEA3* encodes an LRR-receptor-like protein while *FEA4* encodes a bZIP transcription factor. Our analysis showed a strong synergistic interaction between *rel2* and *fea3*, and *rel2* and *fea4*, while an epistatic interaction was detected between *rel2* and *td1* mutants. These results suggest that *REL2, FEA3*, and *FEA4*, work in parallel pathways regulating meristem size, while *TD1* and *REL2* may work in the same pathway. As the signaling cascades initiated by *TD1* and *FEA3* ultimately repress the expression of the stem-cell promoting transcription factor *WUS1*, we are currently analyzing the genetic interactions between *rel2, relk1*, and *wus1* mutants.
We acknowledge funding from the National Science Foundation (IOS#2026561).

Gene / Gene Models described: *REL2, RELK1, RELK2, RELK3, TD1, FEA3, FEA4, WUS1*; GRMZM2G042992, GRMZM2G316967, GRMZM2G030422, GRMZM2G550865, GRMZM2G300133, GRMZM2G166524, GRMZM2G133331, GRMZM2G047448
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P22

Glossy2 and *Glossy2-like* demonstrate both unique and overlapping functions in maize cuticular wax formation.

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The cuticle is a hydrophobic barrier that covers all surfaces of the aerial organs of land plants. It provides the first line of defense from biotic and abiotic stresses that negatively impact plant health. The cuticle is composed of solvent-extractable cuticular waxes that are both intercalated within and laid atop an insoluble cutin polyester matrix. Cuticular wax composition varies depending on organ and stage of development, but usually consists of combinations of very long chain fatty acids (VLCFAs) and their derivatives, including hydrocarbons, alcohols, aldehydes, ketones, and wax esters. Classical genetic strategies have identified numerous *glossy* genes required for normal cuticle deposition in maize, and molecular characterization of these genes provides insights on cuticle formation. This study focuses on the maize *Glossy2* (*Gl2*) gene. Although the biochemical function of GL2 remains unclear, homozygous *gl2* mutant seedlings exhibit a *glossy* phenotype and the cuticular waxes of mutant plants are of shorter chain lengths, presumably due to an alteration of the fatty acid elongase complex (FAE). The Recently identified GL2-paralog, GLOSSY2-LIKE, shares 63% amino acid similarity with GL2. To assess the *in planta* function of *Gl2-like,* six mutant alleles were generated via CRISPR-Cas9 genome editing. Cuticle analyses of single *gl2* and *gl2-like* mutants, and *gl2;gl2-like* double mutants demonstrate both overlapping and distinct contributions to FAE across different tissue types. While cuticular wax composition on maize leaves is primarily affected by the *gl2* mutation, a chemotype was only observed on maize silks in the *gl2;gl2-like* double mutant. The functionality of GL2 and GL2-LIKE proteins has also been investigated via a synthetic biology approach in yeast, in which each paralog was co-expressed in an assortment of strains that also expressed enzymes from the maize FAE system. This combination of multidisciplinary strategies is unraveling the roles that *Gl2* and *Gl2-like* serve in maize cuticle biosynthesis.

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P23

High-sensitivity transcriptional profiling of maize root tips via nascent and nuclear RNA sequencing

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The application of high-throughput sequencing to transcriptome profiling (RNA-Seq) has enabled significant advances in our understanding of gene expression in plants. The most widely adopted RNA-Seq protocol involves the purification of total cellular RNA, followed by reverse transcription and sequencing. Conventional RNA-Seq suffers some limitations. It reflects the balance between synthesis and degradation of each transcript ("steady-state") and not its transcription rate in the nucleus. It also lacks the sensitivity to detect unstable and low-abundance RNA species, such as long noncoding RNAs (lncRNAs). To get a better estimate of transcriptional activity, we used the cell-permeable uracil analog, 5-ethynyl uridine (EU) to label nascent RNA of maize B73 root tips *in vivo* . After ribosomal RNA depletion, the EU-labeled RNA was sequenced, together with the steady-state nuclear RNA and steady-state cellular RNA. The nascent and steady-state cellular RNA profiles were clearly distinct, while the nascent and steady-state nuclear RNA profiles were tightly correlated. About 60% of the expressed genes were differentially expressed (adjusted p-value <0.01) in the cellular vs. nuclear RNA comparisons (median logFC=-13.6, median $\log_{10}$(p-value)=1.67). In contrast, only 20% of the expressed genes were differentially detected in the EU-labeled RNA vs. steady-state nuclear RNA comparisons (median logFC=-3.98, median $\log_{10}$(p-value)=0.64). Both nascent RNA and steady-state nuclear RNA analyses displayed higher sensitivity, notably for the detection of lncRNAs and microRNAs (3-fold and 20-fold higher, respectively, than steady-state cellular RNA). Our results indicate that EU-labeled and steady-state nuclear RNA provide a more accurate and sensitive measure of the transcriptional activity than steady-state cellular RNA, especially for low-abundance and nucleus-specific RNA species. These findings open the possibility of improving transcriptome profiling in maize tissues that are difficult to label, such as aerial parts, or with lower transcription rates than root tips.

Funding acknowledgement: National Science Foundation (NSF)

P24

Identification of genes associated with phenolic metabolism

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Improved genetics and agronomic practices have augmented maize productivity to meet the demands of a rapidly growing population. However, the process of selective breeding applied to increase productivity has resulted in the unintended selection of crop varieties lacking important compounds, such as many phenolics including flavonoids and phenylpropanoids, which play important roles in plant protection against abiotic and biotic stresses and are also beneficial for the human and animal diet, when accumulating in the seed. As a result, this can lead to significant productivity losses under stress conditions. Consequently, there is a growing interest in better understanding the biosynthesis and regulation of phenolic compounds in maize. The objective of this study is to determine genes leading to the formation and regulation of phenolic compounds in maize by conducting two different strategies: (1) Homology-base identification. We utilized the repertoire of well-characterized *Arabidopsis* phenylpropanoid and flavonoid metabolism genes to identify candidate orthologs (homology >90%) in the maize B73 (v4) genome. The protein sequences of these orthologues were used to search the maize genome for similar proteins using BLASTP and a total of 249 gene models from 28 gene families were identified. Then we used available genome-wide expression analysis to determine which family members are predominantly expressed and explored their co-expression with other pathway genes to establish which isoform might participate in the biosynthesis of the various pathway products. (2) A Genome-Wide Association Study (GWAS) for a set of 33 phenolic compounds profiled across 597 genetically diverse inbred lines, grown under controlled conditions. Three biological replicates were used, and seedling stem samples were collected for analysis. Phenolic compounds were quantified using liquid chromatography coupled with mass spectrometry (LC-MS) using a rapid separation targeted method. Our initial results revealed several candidate genes belonging to various families, including UDP glycosyltransferases, acyl-CoA synthetases, 2-oxoglutarate-dependent dioxygenases, and methyltransferases, which are potentially involved in the formation of various phenolic compounds. Additionally, new members of the MYB and BHLH transcription factor families, previously reported to regulate the production of flavonoids, production were identified. A subset of these candidate genes is currently being experimentally validated by monitoring metabolic changes of phenolic compounds in maize protoplasts transformed with the respective genes in various maize genotypes. The outcomes of this study will provide a valuable resource for improved plant breeding for stress tolerance and overall maize improvement.

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P25

Identifying physiologically relevant transcriptional regulatory mechanisms in tetrapyrrole biosynthesis using natural and induced variation in Mg^{2+} chelatase in maize.

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Mutants with defects in pathways can help expose regulation. But strong phenotypically impactful mutations do not always detect the same changes in expression affected by the natural variants that fine tune pathway functions and determine fitness. This is especially true for essential genes and genes in highly conserved pathways. Tetrapyrroles are macrocyclic compounds present in and required for all life on earth. In plants, they include chlorophyll, heme, siroheme, and phytochromobilin and are required for photosynthesis, respiration, metabolism, and light perception. All tetrapyrroles in plants are synthesized from a common phototoxic precursor, Uroporphyrinogen III. Competition among the necessary branches for shared substrates and the presence of multiple phototoxic intermediates and side products suggest that regulation of tetrapyrrole biosynthesis should be tight and complex. Maintaining a balance between the branches to serve the demands of photosynthesis, respiration, and metabolism while avoiding photodamage must be a critical function of this regulation. We used RNA-seq to study the impact of a semi-dominant dominant-negative complex-poisoning allele of Mg chelatase subunit I, *Oil yellow1-N1989*, on transcription in tetrapyrrole biosynthesis pathway. Coordinate and compensatory effects were observed at multiple genes encoding steps in tetrapyrrole biosynthesis demonstrating heretofore unknown transcriptional regulation of this pathway. Expression level GWA analysis of tetrapyrrole pathway genes revealed that natural variation at the *oy1* locus also affected coordinate transcriptional regulation of multiple genes encoding steps in tetrapyrrole biosynthesis in wildtype plants. This transcriptional feedback in the absence of strong phenotypic impacts demonstrates that detected regulation works under physiologically relevant perturbations of chlorophyll biosynthesis. We identified a subset of genes in tetrapyrrole pathway as the common targets of transcription regulation and identified multiple trans-regulatory hotspots affecting the coordinate regulation of this pathway.

Gene / Gene Models described: *oy1*; Zm00001eb407780, GRMZM2G419806, Zm00001d023536
Funding acknowledgement: National Science Foundation (NSF), United States Department of Agriculture (USDA), Department of Energy (DOE)

P26

Identifying the functional determinants of ZmEhd1 - a putative maize flowering time activator using a protoplast system.

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With the human population increasing, it is essential to find novel ways to increase sustainable food production. Maize is a globally important staple crop, but due to flowering time issues, we are not able to fully utilize the extensive genetic diversity of tropical maize for crop improvement. Tropical maize has the potential to contribute desirable traits such as pest/disease resistance, stalk stability, and drought tolerance to temperate breeding efforts, but its late flowering in temperate environments limits its use. Plant biotechnology strategies can be used to address this issue, but first we need to more fully understand the molecular genetic network controlling maize flowering time. My project aims to understand the molecular mechanisms of the maize flowering time promoter, ZmEhd1. ZmEhd1 is a homolog of the rice Ehd1 flowering activator and encodes a B-type response regulator, similar to transcriptional activators of the cytokinin signaling pathway. I am using a maize protoplast system to (i) confirm the correct ZmEhd1 gene structure using rapid amplification of cDNA ends (RACE) (ii) determine if ZmEhd1 localizes to the nucleus, (iii) use deletions to map the ZmEhd1 nuclear localization domain, and (iv) use transcriptomics to determine how overexpressing ZmEhd1 impacts other parts of the flowering time network. Understanding the functional determinants of ZmEhd1 will provide a more complete picture of its role in the maize flowering time network. This new information can help inform novel biotechnological approaches to amend the flowering issues in tropical maize, allowing its broader use in maize breeding programs worldwide, thus, helping to improve sustainable food production.

Gene / Gene Models described: *ZmEhd1*; Zm00001d032784
Funding acknowledgement: National Science Foundation (NSF)

P27

Improving transformation and regeneration of tropical inbred lines using calcium channel blockers

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Commercial production of transgenic maize relies on agrobacterium-mediated transformation due to the simplicity, cost-effectiveness, and stable inheritance of exogenous genes with low copy numbers. However, transformability and rengenerability remain a bottleneck in tropical maize lines, where limited genotypes and lower transformation efficiencies persist. Recent advances in morphogenic regulators (e.g. BBM and WUS2) which enhance somatic embryo formation have expanded the range of transformable genotypes, particularly in temperate maize lines. To address the challenges in tropical maize transformation, our study aimed to enhance the process using calcium channel blockers (CCB) in conjunction with morphogenic regulators. CCBs are pharmacological agents that can hinder the plant's immune response by specifically targeting calcium signaling pathways, affecting hypersensitive response. Here, we investigate the impact of Ca2+ chelators (EDTA and EGTA) and a non-selective calcium channel antagonist, (lanthanum chloride), on the transformability and regeneration frequency of tropical inbred lines, Tzi8 and CML277, using varying concentrations (0, 0.1, 1, 10, 100 mM). We pre-treated immature embryos of Tzi8 and CML277 with calcium channel blockers before transformation with an auxotrophic agrobacterium LBA4404Thy-containing pHP81430, a heat shock excisable BBM and WUS2 vector with a modified acetolactase synthase gene as a selectable marker. Endpoint PCR was used to identify T0 plants and screen for BBM and WUS2. ddPCR was used to determine copy number variation of the T0 plants. The highest regeneration percentages (2.6%) and occurrence of single copy events (3.3%) were achieved in Tzi8 when immature embryos were pre-treated with 10 mM LaCl3. In CML 277, regeneration percentages were highest in immature zygotic embryos pre-treated with 1 mM EDTA (86%), and occurrences of single copy events were at 2.7%. No T0 plants were produced in the no pre-treatment controls for both inbreds. This study successfully improved the transformation and regeneration efficiency of the two tropical inbreds using CCBs.

Funding acknowledgement: National Science Foundation (NSF)

P28 @tinoco_morais

Inoculation effect of phosphate-solubilizing bacteria on the microbiota of maize cultivated under different phosphate fertilization conditions

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Plant Growth Promoting Bacteria (PGPB) are frequently utilized in agricultural settings as bioinoculants due to their positive impact on plant growth. BiomaPhos® stands out as the first Brazilian commercial bioinoculant, featuring a consortium of two P-solubilizing Bacillus strains (CNPMS B2084 and CNPMS B119). Our objective was to assess the influence of BiomaPhos® inoculation and phosphate fertilization on the microbial communities within the maize rhizosphere. Field experiments were conducted at the Embrapa Maize and Sorghum Experimental Station in Sete Lagoas, Minas Gerais, Brazil, using clayey soil (Oxisol) during the 2019/2020 and 2020/2021 seasons. The maize genotype DKB390 underwent inoculation with and without BiomaPhos® and was subjected to three P-fertilizer treatments: no P-fertilizer addition (P0), Rock phosphate (RockP), or triple superphosphate (TSP) at a rate of 120 kg of P2O5 ha-1. Genetic diversity was evaluated during flowering time through T-RFLP, and taxonomic groups were identified using MiCA3 software. In the 2019/2020 harvest, no significant differences in bacterial diversity were observed among treatments. However, in 2020/2021, notable distinctions in bacterial communities emerged between the rhizosphere soil of inoculated and non-inoculated maize crops cultivated with 120 kg.ha-1 of TSP and P0. The predominant bacterial families in the first and second seasons were Streptomycetaceae (34.6% and 40.4%), Micrococcaceae (15.14% and 12.0%), and Methylobacteriaceae (10.9% and 13.7%), respectively. Additionally, there was a noteworthy increase in the abundance of the Rhizobiaceae, Sphingnomonadaceae, and Brucellaceae families, coupled with a significant reduction in the relative abundance of Clostridiaceae, Geobacteraceae, Micrococcaceae, and Pseudomonadaceae in the second season. The comprehension the impact of inoculation should be further investigated. Further exploration is needed to understand the implications of inoculation.

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

P29

Insights into genomic regions associated with resistance and susceptibility to Gibberella ear rot

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Phytopathogenic fungi are some of the most damaging plant pathogens. In maize, ears can be colonized and infected with the fungus *Fusarium graminearum,* the causal agent of Gibberella ear rot (GER). Infected grain contains mycotoxins that causes severe health risks to humans and livestock when consumed. Host resistance is an important management strategy for GER. In 2021 and 2022 we screened a diverse panel of maize lines (n= 318) for resistance and identified markers associated with GER resistance and toxin accumulation. We then selected and screened near-isogenic lines based on our findings and the literature regarding GER resistance. The selected near-isogenic lines are either in a B73 background with introgressions from teosinte (n= 25) or Oh43 (n= 72), or in an H100 background with introgressions from NC344 (n= 202). We evaluated these lines for resistance to GER in 2023 in Urbana, IL. In the B73 × Oh43, B73 × teosinte, and NC344 × H100 populations most lines were moderately resistant with a slight skew towards susceptibility. We identified multiple lines that were significantly different ($\alpha = 0.05$) from their recurrent parent based on a Dunnett's post hoc test. A total of 6 and 3 lines were significantly more susceptible than B73 in the B73 × Oh43 and B73 × teosinte populations, respectively. In the NC344 × H100 population, 14 lines were significantly more resistant than NC344. We then identified regions associated with susceptibility and resistance. We plan to evaluate the selected near-isogenic lines again in 2024 to validate our findings. We believe that these lines will be useful for exploring the mechanisms underlying susceptibility to GER.

Funding acknowledgement: United States Department of Agriculture (USDA)

P30

Integration of proteomics and phosphoproteomics provides insight into the protein-bound amino acid regulation in the opaque2 mutant

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Cereal Grains are a poor source of essential amino acids (EAA). A diet that lacks a sufficient quantity of EAAs contributes to malnutrition and growth deficiencies. As most amino acids in the seed are constituents of proteins, targeting protein-bound amino acid (PBAA) for nutritional enhancement is the most plausible method. However, previous studies have attempted to overexpress or knockout proteins to increase EAAs contents, but it was largely unsuccessful. This is because PBAA regulation is a complex trait that is tightly controlled by the interactions of multiple networks in the cell. The tight maintenance of PBAA composition and proteome reprogramming in mutants of seed storage proteins is shared across multiple organisms. However, we know very little about the molecular mechanism underlying the regulation of PBAA in the seed. To better understand the genetic and metabolic basis of PBAA robustness and regulation in the seed, we have utilized a combination of mutant approaches and multi-omics by performing differential proteomics, phosphoproteomics, and transcriptome-wide association study (TWAS) on seed storage protein mutants and compared them to unperturbed genotypes. Overlap of TWAS and differential proteomics identify 101 high-confidence candidate genes mostly involved in protein metabolism. From the overlap, we saw a significant enrichment of translational components. Changes in protein abundance and gene expression show heterogeneous expression of translational components across development across tissue types and between genotypes. Phosphoproteomics results show that change in phosphorylation is independent of change in protein abundance. Phospho-peptide enrichment at 26DAP shows an overall increase in the phosphorylation of translation initiation and mRNA binding, while there is a decrease in phosphorylation of mRNA metabolic processes. These results indicate a potential role for the translational machinery in maintaining/regulating protein content and amino acid composition in the seed. Further analyses of translational-component mutants will provide more insight into the changes in PBAA and FAA across seed development. Understanding the tight regulation of PBAA will enable us to find new strategies to push forward amino acid biofortification efforts.

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P31 @minorallele

Introgression of a highland maize chromosomal inversion decreases flowering time in a phosphorus-independent manner, and leads to minor perturbations of the leaf phosphorus starvation response transcriptome.

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Inv4m is a chromosomal inversion commonly found in traditional maize varieties grown in the Mexican highlands. Inv4m was introgressed into cultivated maize from highland teosinte mexicana, and its highland predominance points to its contribution to local adaptation. In a Mexican diversity panel, Inv4m shows a classical pattern of gene-by-environment interaction produced by local adaptation: plants with the Inv4m-highland allele have delayed flowering in lowland fields and earlier flowering in highland fields. In growth chamber experiments, Inv4m-highland has been shown to regulate the expression of photosynthesis genes in response to cold. In addition to cold, phosphorus is a limiting factor for plant growth in the Mexican highlands. In these conditions, Inv4m might increase highland fitness by carrying beneficial alleles of phos2 a locus involved in phosphorus starvation response (PSR). In this study, we test whether Inv4m-highland contributes to local adaptation through an enhanced response to phosphorus deficiency. First, we bred Near Isogenic Lines (NILs) in B73 containing Inv4m-highland introgressed from MICH21, a traditional Mexican maize variety, for isolating the effect of Inv4m-highland in a single genetic background. Then we grew the Inv4m-highland and control lines in the field under phosphorus sufficiency and deficiency. We measured flowering time, morphological traits, and leaf gene expression with RNA-seq. We found that independent of soil phosphorus status, Inv4m-highland NILs flowered faster and grew taller than the controls while maintaining grain yield. There was a genomewide transcriptomic response to available phosphorus, affecting 10978 differentially expressed genes (DEGs), with the largest effect shown by PILNCR1-miR399, a PSR regulator. The effect of Inv4m-highland is narrower, affecting 619 DEGs, mainly within the boundaries of the inversion. The Inv4m perturbation of PSR is limited to 157 DEGs. Our results confirm the contribution of Inv4m to adaptation, as faster flowering, but we don't find evidence of its dependency on PSR.

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

P32

Investigating the expression regulation and enzymatic activity of the adaptive gene hpc1

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After domestication, maize adapted to very different environmental conditions across the world. The molecular basis of how maize was able to adapt is not fully understood. Our lab has recently reported that variation in phosphatidylcholine levels in Zea mays helped maize adapt to different elevations. Variation in phospholipid levels from maize varieties adapted to different elevations is largely attributed to High PhosphatidylCholine 1 (HPC1), which encodes a phospholipase A1 enzyme (Barnes, Rodríguez-Zapata, Juárez-Nuñez et al. 2021). Palomero Toluqueño (PT), a highland traditional variety from central México, and other highland varieties including the highland teosinte mexicana carry an impaired hpc1 function. The highland allele has also been found in maize adapted to colder environments, like Canada and Northern Europe. Maize lines carrying the highland hpc1 allele have shorter flowering times and better fitness when grown in highland conditions when compared to lowland maize. However, the molecular mechanisms of hpc1 impaired function are still unclear. On one hand, a mutation in the flap lid domain of hpc1 in highland maize may impair phosphatidylcholine access to the enzyme's active site. On the other hand expression data and promoter sequence analysis suggest that variation in hpc1 expression levels may also play a role in determining the metabolic output of hpc1 genetic variation To investigate different genetic elements and enzymatic activity between HPC1-PT and HPC1-B73, we have cloned and expressed HPC1 in yeast. Using this heterologous system, together with targeted mutagenesis and lipase domain swapping between the two hpc1 alleles we hope to identify the possible causative mutations affecting HPC1 activity. . Additionally, we are performing promoter swapping between the B73 and PT alleles of hpc1 to explore the possible effect of hpc1 regulatory variants on hpc1 expression and the possible interaction between hpc1 gene expression and HPC1 enzyme activity.

Gene / Gene Models described: *HPC1*; Zm00001121780
Funding acknowledgement: National Science Foundation (NSF)

P33 @dsegovia93

Investigating the maize systemic disease response to the southern leaf blight pathogen, *Cochliobolus heterostrophus.*

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Plants rely on cell-mediated innate immunity and systemic signals from infected sites to activate defense responses against pathogens. The plant's local response to infection induces the production of signaling compounds such as salicylic acid that will further lead to the systemic expression of pathogenesis-related (PR) genes in a phenomenon called systemic acquired resistance (SAR). Consequently, the systemic acquired resistance response results in enhanced broad-spectrum immunity of distantly located tissues. Although research on SAR in monocotyledonous systems has increased over the years, most of the research has been conducted on dicotyledonous plants. Maize is an important staple food worldwide and it can be highly affected by diseases causing yield losses and reduced food availability, thus affecting food security. This study aims to investigate the maize SAR response to the southern leaf blight (SLB) pathogen, *Cochliobolus heterostrophus,* which is found in maize cultivation all around the world. In our preliminary experiments, a series of 3-week-old B73 maize plants showed that a localized inoculation with *Cochliobolus heterostrophus* at different time points (time 0, 24, and 48 hours) in the 3rd, 4^{th,} and 5th leaves resulted in less disease severity in the upper leaves of inoculated plants when compared to the non-inoculated plants 72 hours post-inoculation. Further analysis on different maize lines will be conducted to confirm that *Cochliobolus heterostrophus* induces the SAR response and how it differs between lines.

Funding acknowledgement: United States Department of Agriculture (USDA), Fulbright IIE, DEPP NCSU

P34

Investigating the role of the cis-regulatory modules and hopscotch in maize domestication QTL teosinte branched1

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Plants contain a multitude of cell types, yet all these cells arise from the same DNA. Correct spatiotemporal regulation of gene expression is crucial for the production of specialized cell types. This spatiotemporal gene expression is mediated by DNA sequences known as cis-regulatory elements (CREs). CREs, often present in clusters, recruit transcription factors (TFs) to activate or repress local gene expression. In this way, CRE clusters can be classified into two broad categories: enhancers and silencers. Enhancers increase the expression of nearby genes irrespective of the orientation, direction, and distance. Conversely, silencers reduce the expression of the target genes. Some silencer CREs recruit the Polycomb Repressive Complex2, which deposits the transcriptionally repressive histone modification H3K27me3 at nearby sequences. Teosinte branched 1 (Tb1) is one of the major domestication QTL controlling branching in maize. Tb1 is responsible for suppression of axillary bud outgrowth on the main stem and development of female inflorescence in maize. Tb1 expression is controlled by a H3K27me3 regulated distal control region. This Tb1 regulatory region exhibits tissue-dynamic chromatin accessibility and H3K27me3 levels that correspond to Tb1 activation, making it a good model to study how plant CREs regulate histone modifications to control gene expression. This study dissects the roles of the TB1 distal regulatory region by using CRISPR-CAS9 to systematically delete the underlying CREs and determine their roles in controlling TB1 expression. Preliminary phenotypic results showed that Tb1 deletion and specific CRE deletion events produced morphological effects such as tillering and masculinization of the maize ear. Extensive Phenotyping of all the deletion lines is currently being performed as well as understanding how distinct CREs within the Tb1 QTL interact with one another.

Gene / Gene Models described: *tb1*; Zm00001d033673
Funding acknowledgement: National Science Foundation (NSF), United States Department of Agriculture (USDA), United Soybean Board

P35

Investigation of tissue-specific translation regulation by uORFs in maize

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Understanding the molecular mechanisms that modulate protein abundance will allow breeders to use modern gene editing and standard breeding techniques effectively for generating more resilient and productive varieties for the challenging environments to come with the new global climate. One such mechanism is protein abundance regulation by upstream open reading frames (uORFs) in the 5' untranslated regions (UTRs) of messenger RNAs (mRNAs). uORFs are prevalent among eukaryotes. Their start and stop codons appear to be evolutionarily more conserved than expected by neutral evolution suggesting that their repressive activity is important for adequate translation. Evidence for this is seen when mutations that modify existing uORFs or create new ones are known to cause diseases in humans. In plants, uORFs have been found to affect phosphorus content in leaves, petal color, and grain dimension for example. In fruit fly, regardless of the rate of transcription, uORFs show varying patterns of ribosome occupancy across tissues for each transcript. This suggests that uORFs are translated in a tissue-specific manner. We gathered ribosome profiling and mRNA sequencing data from 44 samples in maize (22 tissues and 2 biological replicates) to determine if uORFs are also translated in a tissue-specific manner. We identified 131,177 candidate uORFs with canonical start (i.e., AUG) and stop (UAA, UAG, and UGA) codons. We calculated the expression as transcript per million (TPM) for the uORFs and the mORFs (i.e., coding sequences) for each transcript. We find, as in fruit fly, that uORFs have different translation rates among different tissues. We have calculated the Pearson correlation coefficient of the rate of ribosome occupancy in the uORFs versus that in the mORFs. We are using this relationship to identify uORFs that are repressing translation and to investigate patterns of global protein abundance regulation.

P36

MOA-seq identifies candidate cis-elements associated with response to submergence.

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Chromatin accessibility to diffusible nucleases such as DNase I and MNase has long been accepted as a feature of open chromatin regions in eukaryotic genomes. These open chromatin regions are generally transcriptionally active, enriched in gene-regulatory sequences, and revealed using these nucleases under partial digest conditions, which serve to preferentially release DNA fragments for analysis. These nuclease hypersensitivity-defined sites frequently contain specific DNA sequences known as *cis*-acting regulatory elements (CREs), including transcription factor (TF) binding sites. The properties of MNase allow for high-resolution mapping of chromatin particles which mostly consist of two components, a sequence-specific DNA-binding protein and its cognate CRE. Therefore, analysis of small fragments of DNA recovered from partial or light chromatin digests enables single-assay genome-wide identification of nuclease-protected footprints of hundreds of TFs within accessible chromatin regions. These footprints represent a transcriptional regulatory atlas of TF-occupied sites, collectively referred to as a cistrome occupancy map. Cistrome occupancy can vary across cell or tissue types in a given eukaryotic genome, as well as across various stress states. By comparing cistrome occupancy across physiological states, one can identify candidate CREs for control of transcriptional responses to environmental or developmental cues. This study applies our established MNase-defined cistrome-Occupancy Analysis (MOA-seq) to a set of flood-treated maize seedlings with variation in a previously characterized flood-tolerance phenotype. Two resistant (Oh7b and Ky21) and one sensitive (B73) inbred maize lines, along with the closely-related grass, *Sorghum bicolor* (BTx623), were subjected as young seedlings to 24 hours of total underwater submergence. For each genotype, MOA-seq and RNA-seq analyses revealed hundreds of genes that underwent significant changes in transcript abundance and identified several thousand loci that exhibited changes in small particle occupancy. Together, these analyses reveal atlases of *cis*-elements implicated in genomic response to submergence underlying variation in an abiotic stress tolerance phenotype.

Funding acknowledgement: Bayer Grants4Traits

P37

Maize cytoplasm genotype is a key determinant of transformability

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Genetic transformation is a process by which novel DNA and or proteins are introduced into crops and has been an essential tool for improving crop performance via genetic engineering and genome editing. One of the biggest challenges of maize transformation is identifying an inbred that is commercially relevant and transformable. Over the past 20 years, several studies have identified nuclear loci that are associated with transformability in maize, but these factors are generally unable to impart transformable broadly across germplasm. One unexplored area contributing to maize transformation is mitochondrial genotype. There are five cytoplasm genotypes in maize characterized by both differences in sequence and their impact on pollen production in combination with nuclear encoded genes. The two "normal" cytotypes (normal A and normal B) have no know impact on any biological process while cms-C, cms-S and cms-T contain unique mitochondrial open reading frames that require nuclear encoded gene products to overcome their impacts (Allen et al. 2007). Here, approximately 100 maize inbred lines were screened for cytoplasm genotype, and six Normal A cytotype inbreds were confirmed to be transformable. These studies demonstrating that a fast and simple screening for cytoplasm genotype has the potential to advance crop engineering by using superior corn germplasm for transformation.

P38

Maize Terpene Synthase 1 impacts insect pest behavior via the production of monoterpene volatiles *β*-myrcene and linalool.

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Plant-derived volatiles are important mediators of plant-insect interactions as they can provide cues for host location and quality, or act as direct or indirect defense molecules. The volatiles produced by *Zea mays* (maize) include a range of terpenes, likely produced by several of the terpene synthases (TPS) present in maize. Determining the roles of specific terpene volatiles and individual TPSs in maize-insect interactions is challenging due to the promiscuous nature of TPSs *in vitro* and their potential for functional redundancy. In this study, we used metabolite GWAS of a sweetcorn diversity panel infested with *Spodoptera frugiperda* (fall armyworm) to identify genetic correlations between TPSs and individual volatiles. This analysis revealed a correlation between maize terpene synthase 1 (*Zm*TPS1) and emission of the monoterpene volatiles linalool and *β*-myrcene. Quantification of headspace volatiles in a maize *tps1* loss-of-function mutant confirmed that *Zm*TPS1 is an important contributor to linalool and *β*-myrcene emission in maize. Furthermore, pairwise choice assays between *tps1* mutant and wild-type plants showed that *Zm*TPS1, and by extension its volatile products, aid host location in the chewing insect *S. frugiperda,* yet repel the sap-sucking pest, *Rhopalosiphum maidis* (corn leaf aphid). *Zm*TPS1 is therefore an important mediator of the interactions between maize and its insect pests.

Gene / Gene Models described: *ZmTPS1*; GRMZM2G049538
Funding acknowledgement: National Science Foundation (NSF), United States Department of Agriculture (USDA)

P39 @PlantaAurelius

Maize-specific LOX4 and LOX5 genes subfunctionalize to play contrasting roles in defense against necrotrophic and hemibiotrophic pathogens

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Anthracnose Leaf Blight (ALB) and Stalk Rot, caused by a hemibiotrophic fungal pathogen, *Colletotrichum graminicola*, results in maize annual losses of up to $1 billion in the United States. Additionally, the necrotroph *Cochliobolus heterostrophus* causes Southern Corn Leaf Blight (SCLB) disease, which devastated 15% of US and southern Canada maize crops during the 1970 epidemics, and results in substantial yield loss to this day. To improve resistance to these pathogens, a better understanding of the molecular mechanisms of defense and new genetic targets for molecular breeding are needed. Fatty-acid derived oxylipins, mainly the products of lipoxygenase (LOX) pathway, are implicated in defenses against pests and pathogens. Here, we report the identification of two non-JA producing, tonoplast-localized 9-LOXs, ZmLOX4 and ZmLOX5, that share 94% amino acid sequence identity to each other and play unique roles in defense to these pathogens. Remarkably, *lox4* knock-out mutants are susceptible to ALB but resistant to SCLB, whereas *lox5* mutants display the exact opposite phenotypes; they are resistant to *C. graminicola*, but susceptible to SCLB. These results suggest that the two duplicated paralogues evolved to specialize in conferring defense to these two pathogens with contrasting disease lifestyles. Metabolite analyses revealed that such functional divergence can be explained by the differential role of these highly related enzymes in the regulation of biosynthesis of the major defense hormones, salicylic acid and jasmonic acid, in response to infection by these two pathogens.

Gene / Gene Models described: *LOX4, LOX5*; Zm00001eb054050, Zm00001eb216870
Funding acknowledgement: United States Department of Agriculture (USDA)

P40

Maize *rough endosperm6* (*rgh6*) encodes a predicted DEAD-box RNA helicase and affects miRNA processing in endosperm development

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Maize *rough endosperm* (*rgh*) mutants have defective kernels with a rough, etched, or pitted endosperm surface. Molecular genetic analysis of this mutant class has identified multiple RNA processing proteins critical to endosperm development. Here, we report on the developmental and molecular function of the *rgh6* locus. The *rgh6* mutant was isolated from the UniformMu transposon tagging population. Mutant kernels have reduced endosperm size and defective embryos that develop in a more apical position than typical for defective embryos. TB translocation crosses revealed that *rgh6* mutant endosperm inhibits normal embryo development. Positional cloning of the *rgh6* locus found that it encodes a predicted DEAD-box RNA helicase. Consistent with a predicted function for RNA processing, transient expression of a RGH6-GFP fusion protein is localized to nucleolus and nuclear speckles in *Nicotiana benthamiana* leaves. *Rgh6* transcripts are highly expressed in endosperm epidermal cell types such as the aleurone, basal endosperm cell layer, embryo surrounding region, and endosperm adjacent to scutellum. Markers of these cell types show increased levels in *rgh6* mutant kernels. Mutant endosperm tissues have increased precursor microRNA (pre-miRNA) and decreased mature miRNA relative to normal sibling endosperm, indicating that *rgh6* is required for miRNA processing. The transcript levels for most miRNA target genes accumulate to a higher level in *rgh6* mutant tissue. These results suggest that miRNA processing and regulation of miRNA target genes are required for normal endosperm development.

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

P41

Minus directed kinesin allows chromosome 10 haplotypes to compete

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85% of the maize genome is comprised of repeat elements of which 9.4% are tandem repeats called knobs. This equates to about ~1.5 Arabidopsis genomes of exclusively knob sequence. These knobs come in two classes (TR1 and knob180) differentiated by their repeat sequence. Abnormal chromosome 10 (Ab10), a large selfish variant of chromosome 10, utilizes knob sequence to be preferentially transmitted to the next generation. Ab10 encodes both knob repeats as well as two kinesin proteins: kindr and trkin. kindr associates with knob180 knobs and trkin associates with TR1 knobs. kindr is responsible for the majority of Ab10 preferential transmission while the role of trkin is unclear. Several hypotheses have suggested that trkin may be improving the fitness of Ab10 in some way. We found that the frequency of meiotic errors did not differ between Ab10 homozygous plants with and without trkin. This suggests that trkin is unlikely to be improving Ab10 fitness by preventing meiotic errors in a meaningful way. Additionally, competition assays between variants of Ab10 demonstrated that trkin is responsible for the ability of K10L2, a trkin bearing N10 like chromosome 10 variant, to compete with Ab10. These data suggest that trkin and kindr may represent independent drive systems capitalizing on the same haplotype.

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P42

Modular base editing

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CRISPR-Cas systems provide powerful molecular toolboxes allowing precise changes in genomes. However, use of those molecular editors needs specific development for use in field seeds crops. Moreover, knock-out of key genes regulating important agronomic traits may be deleterious to plant yield or plant survival. Therefore, it is of interest to improve crops by creating specific allelic versions via precise single-nucleotide modification(s) which would provide trait of interest while retaining basic gene function. Such tools include Base Editors that can mediate substitutions. Amongst commonly used base editors are those associated with a Cas9 nickase to nick the non-edited DNA strand. Typically, the Cas9 nickase is fused to the base editor deaminase domain. Alternatively, it has been shown in rice that the nCas9 deaminase complex can be formed through an indirect interaction of the deaminase with a modified gRNA which then guided the base editor complex to the target. Here we show that the deaminase domain can be provided separately from Cas9 nickase to perform base editing. Although editing level is lower as compared to that obtained using a direct fusion, this strategy allows for a more flexible use of base editing since specific base-editing constructs are not required for use with alternative Cas systems. This approach may be of particular interest when optimizing components for base editing.

Funding acknowledgement: INRAe, Limagrain LFS research

P43

Molecular identification of two *Mu*-suppressible alleles of *lesion28* as insertions in UROPORPHYRINOGEN III SYNTHASE

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Porphyrins are tetrapyrrole required for all known lifeforms. Porphyrins include specialized molecules, including siroheme, cobalamin, heme, bilins, and chlorophyll, required for multiple metabolic processes and adaptative responses to environmental cues. The porphyrin biosynthetic enzymes Porphobilinogen Synthase, Hydroxymethylbilane Synthase, and Uroporphyrinogen III Synthase are conserved across all life on earth. Previously, the semi-dominant lesion-forming *Les28-1* mutant was characterized as a *Mu*-suppressible allele, where lesion formation was increased by increasing the activity state of the *Mutator* transposons in *Les28-1*/+ plants. We extend these observations and demonstrate that *Mu-killer*, which epigenetically silences *Mutator*transposon activity, also suppresses the lesion formation on *Les28-1* plants. Analysis of sequences flanking *Mu*-insertions in *Les28-1* that co-segregate with lesion formation identified an insertion in the first intron of maize uroporphyrinogen III synthase as the cause of the *Les28-1* allele. A second insertion allele, in the 5' UTR of this gene, was obtained from the photosynthetic mutant library at Oregon State University and found to encode a second *Mu*-suppressible allele, *Les28-2,* that resulted in semi-dominant and light-dependent lesion formation and recessive day-night-cycle-dependent necrotic stripe formation on leaves. Allelism tests between the two alleles produced yellow-seedling lethal offspring similar to recessive loss-of-function phenotypes in other steps in the porphyrin pathway. To discover naturally occurring alleles affecting variation in porphyrin metabolism, we scored the lesion phenotype in roughly five-thousand *Les28-1*/+ F1 plants obtained by crossing this mutant to an association mapping population. GWAS and candidate gene association detected epistatic effects on *Les28-1* linked to genes in the porphyrin pathway, including the first committed steps of the heme, siroheme, and chlorophyll branches. This work provides evidence of cryptic standing variation in the maize germplasm via an epistatic interaction with novel genetic perturbation of an evolutionary conserved enzyme.

Gene / Gene Models described: *les28*; GRMZM2G093197, Zm00001d027950, Zm00001eb006450
Funding acknowledgement: National Science Foundation (NSF), United States Department of Agriculture (USDA), Department of Energy (DOE)

P44

Natural variation in maize root hydraulic architecture may offer new insights into plant drought responses

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Water availability is one of major challenges for modern agriculture. This tension is exacerbated by extreme weather events and global climate change. In this context, understanding and controlling plant responses to drought have become crucial.
Roots play essential roles in soil water uptake. The water uptake capacity of roots is determined by both the root system architecture (RSA) and its water permeability (hydraulics), which together shape the root hydraulic architecture. In the present project, we aim to explore the natural genetic variation of maize root hydraulic architecture to identify key molecular and genetic components and investigate their impact on plant drought responses.
We examined root hydraulic conductivity (Lp_r), a measure of root water permeability, in 224 maize Dent inbred lines under non-drought conditions (Rishmawi et al., 2023, Plant Physiol. 192:2404-2418). This revealed significant natural variations in the Lp_r of primary roots during an 11 to 12-day growth period. Genome-Wide Association Studies (GWAS) lead to the identification of 6 QTLs with 8 underlying candidate genes. These genes are involved in many biological pathways such as amino acid metabolism, ethylene signaling, and vascular development.
We took two genetic validation approaches for these QTLs: confirming allelic effects on Lp_r using bi-parental recombinant populations and verifying the function of candidate genes through knock-out mutants created by transposon insertion or CRISPR-Cas9. Our results demonstrate a distinct allelic effect on Lp_r at the identified QTL loci. Furthermore, mutant analysis for select candidate genes suggests their involvement in the regulation of Lp_r in both maize and Arabidopsis roots. Currently, we are investigating the cellular and molecular mechanisms of the candidate genes based on their predicted mode of action. Gene-based association mapping will provide detailed insights into causal allelic variations. Subsequently, we will explore the effects of these alleles or genes under various drought conditions.

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P45

Nutritional and biophysical characteristics of CRISPR-generated low kafirin, waxy sorghum

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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. Here, we report on results from a highly-inbred edited line with ~400kb of the *k1C* family (12 active genes) deleted. These results 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 will provide insight into the nutritional and biophysical properties of sorghum grain produced from this novel genotypic combination.

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

P46 @julianayassitepe1

Phenotyping transgenic events for drought resistance

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Drought and heat stress limit maize growth and development, decreasing annual yield in many regions worldwide. As temperatures continue to rise, tropical regions will be disproportionately affected, adversely impacting the quality and production of agricultural products, requiring integrated management practices and the adoption of stress-resistant varieties to mitigate losses. Maize plants evolved different morphological and physiological mechanisms to grow under water-limited conditions, affecting several agronomic traits, but only a few major genes were reported associated with yield. At the Genomics for Climate Change Research Center (GCCRC), we study both genes of unknown function involved in drought response in extremophile plants and genes of the alternative respiratory pathway from cultivated species. After a significant increase in shoot and root biomass under water restriction in a greenhouse experiment, different events overexpressing the UCP1 (Uncoupling protein 1) from *Arabidopsis thaliana* or an uncharacterized gene (gccrc candidate gene 20) from a halophyte species were tested in the field under well water and drought conditions in two maize genetic backgrounds (B104xCML488 and B104xCML360) during the Brazilian second season in 2023. We observed variation among events in agronomic and yield components for each gene. Some events demonstrated superior performance compared to the controls. In the B104xCML360 background under drought conditions, both genes contributed to an increase in yield components, whereas in the B104xCML488 background, they did not have the same effect. While CML488 is a drought-tolerant line adapted to the Africa CYMMIT program, CML360 is adapted to acid soils from South America. Field phenotyping of additional genes is in progress in the GCCRC pipeline.

Funding acknowledgement: Fapesp - Fundação de Amparo a Pesquisa do Estado de São Paulo

P47

Proteomic and metabolomic landscape of the arbuscular mycorrhizal symbiosis in maize roots

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Phosphorus (P) is the second most important macronutrient for plant growth, and despite being present in soils in significant amounts, its availability to plants is limited due to various pH-dependent complexations with soil minerals. The production of inorganic P fertilizers is highly energy-intensive and is directly tied to the consumption of phosphate rock (PR), a nonrenewable resource that is expected to be depleted within the next 50-80 years. Symbiosis of crops with arbuscular mycorrhizal fungi (AMF) is a promising avenue to enhance phosphorus use efficiency. However, the regulatory mechanisms by which AMF promotes phosphate utilization in plants, especially maize, are largely unknown. To identify the physiological processes and pathways that facilitate the mobilization and uptake of P, we profiled the metabolome and proteome of maize roots grown under axenic conditions with and without AMF association. We show that localized changes (>8 times) in benzoxazinoids, a tryptophan-derived secondary metabolite, and carbohydrate metabolism have a major role in root-AMF interactions. At the proteome level, upregulation of flavin-containing monooxygenase, plant lipid transfer protein, putative sugar-phosphate/phosphate translocator, and SnRK1-interacting protein is associated with the symbiosis. Our study provides a mechanistic understanding of the signaling nexus underlying root mycorrhizae interaction that improves P utilization.

Funding acknowledgement: National Science Foundation (NSF)

P48

Pseudomonas syringae pv. tomato DC3000 induces defense responses in diverse maize inbred lines

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Many phytopathogens translocate virulence (effector) proteins into plant cells to circumvent host immune responses during infection. One such pathogen is Pseudomonas syringae pv. tomato DC3000, which secretes at least thirty-six effectors into host cells, of which a subset elicit host defense responses in crop plant species such as wheat. However, it is unknown whether P. syringae pv. tomato DC3000 is capable of activating immune responses in maize inbreds. We, therefore, screened a diverse maize germplasm collection for effector-dependent recognition of this bacterial pathogen. As a control, we infiltrated Pseudomonas syringae DC3000(D36E), a derivative of P. syringae pv. tomato DC3000 that lacks all endogenous effectors. In our evaluations, we observed a variety of responses to P. syringae pv. tomato DC3000 in maize and scored the phenotypes as either no observable response (N) or as one of three responses: weak chlorosis (WC), chlorosis (C) with minimal cell death, and hypersensitive reaction (HR)-like cell death. Of the twenty-six maize inbreds screened, 13 were scored as N, 2 as WC, 2 as C, and 9 as HR-like cell death. Importantly, no maize line responded to P. syringae DC3000(D36E), demonstrating the phenotypic responses observed are likely dependent upon recognition of one or more P. syringae effectors. Consistent with the phenotypic responses, maize inbred lines that recognize P. syringae pv. tomato DC3000 accumulated detectable hydrogen peroxide within the infiltrated regions as well as an increase in transcript expression of a subset of maize defense genes. Collectively, our results reveal P. syringae induces defense responses in diverse maize inbreds. Our results, therefore, suggest that diverse maize inbred lines likely encode disease resistance proteins that recognize the activities of one or more P. syringae pv. tomato DC3000 effectors.

Funding acknowledgement: United States Department of Agriculture (USDA)

P49

Roles of REL2 mediated transcriptional co-repression in maize immunity

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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 various 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. Lastly, we have created an integrated gene regulatory network with mass-spec-based proteomics and transcriptomics that give insight into how REL2 plays a role in maize immunity. This work aims to elucidate how hyperacetylation impacts the biological activity of REL2 and REL2's roles in plant-pathogen interactions to gain a detailed molecular understanding of plant immunity and to reconstruct a model for how REL2 transcriptionally regulates plant pathogen response.

Gene / Gene Models described: *REL2*; Zm00001d024523
Funding acknowledgement: United States Department of Agriculture (USDA)

P50

Roles of vacuolar invertase genes (Ivr1 & Ivr2) in the pollination biology of maize

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Invertases are essential to developing kernels and other tissues that depend on import of sucrose transported from leaves. These enzymes are pivotal to sucrose and catalyze the first step in its metabolism. In addition, vacuolar invertases can link sucrose import to cellular expansion in growing tissues. The Ivr1 invertase is expressed primarily in male plant parts (anthers and pollen), whereas Ivr2 localizes to female parts (silks) and root tips. Together they can markedly impact pollination success. The Ivr1 gene also shows a "domestication signature" suggesting a selective advantage during ancient breeding and emphasizing its biological significance. The goal of this work is to determine the contributions by each of these invertases to pollination biology of maize. Genetic materials (knock-out mutants) have been developed that will allow individual roles to be tested. Four distinct lines with mutant alleles disrupting the Ivr genes at different sites have been confirmed, two disrupting the Ivr1 gene and two disrupting the Ivr2 gene. These provide a foundation for in-depth exploration. Phenotypical analysis of invertase mutants are ongoing, with preliminary results showing an increased anthesis silking interval and stunted pollen germination in the mutants of both the Ivr1 and Ivr2 genes; two factors pivotal to pollination success. Molecular analyses by PCR and qPCR will parallel field studies to quantify the expression of both genes at crucial sites during pollination, particularly in tissues of rapid cellular expansion, such as the silks and the filaments. In an era of growing demand for sustainable food production, understanding the intricate genetic underpinnings of yields from vital crop species like maize is essential. This research will help dissect complex genetic interactions that sustain a crop that feeds the world, with far-reaching implications for agricultural innovation and global food security.

Gene / Gene Models described: *Ivr1, Ivr2*; GRMZM2G394450, GRMZM2G089836
Funding acknowledgement: National Science Foundation (NSF)

P51

Site-directed integration of microbial *HemG-type protoporphyrinogen IX oxidase* (PPO) at a target site in corn genome creates tolerance to PPO-inhibiting herbicides

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Crop herbicide tolerance traits are important for weed management and crop yield. However, continuous herbicide application has driven weeds to evolve herbicide resistance which challenges corn production. Therefore, creating new herbicide tolerance trait is essential to reduce herbicide resistance evolution in weeds and sustain crop yield. Expression of a microbial HemG-type protoporphyrinogen IX oxidase, PPO_H_N90, in crops enabled tolerance to PPO-inhibiting herbicides in Bayer. In this study, PPO_H_N90 was inserted to a pre-selected target site in corn genome by Site-Directed Integration (SDI) technology. After several rounds of self-pollination and selection, one lead event was identified to contain a single, intact and full-length PPO_H-N90 gene at the target site without backbone and SDI machinery, and PPO expression was detected in the lead event. In field trials, the lead event showed excellent tolerance to PPO-inhibiting herbicides, while the agronomic traits were not affected by PPO expression and application of PPO-inhibiting herbicides.

P52

Systematic exploration of transcription factor function in maize

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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's 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), NIH T32

P53

Tar spot and fisheye lesions exhibit distinct yet overlapping transcriptomic signatures, suggesting a complex interplay of shared and specific pathways modulated by *Phyllachora maydis.*

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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)

P54

Targeting meiotic crossovers in maize

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Controlling meiotic recombination will allow utilizing larger numbers of allele combinations in breeding programs and make plant breeding more efficient. To do it, we are developing methods to stimulate recombination at specific genome loci. Since meiotic recombination starts with double-strand breaks (DSB) introduced into chromosomal DNA by the SPO11 protein complex, we target the SPO11-1 protein to defined sites in the maize genome using the CRISPR/Cas9 technology. In this approach, SPO11-1 fused to a catalytically inactivated dCas9 protein is directed using guide RNAs (gRNAs) to the targeted loci, where it is expected to generate DSBs. Increasing DSB frequencies should lead to higher crossover numbers. To test the efficiency of DSB formation, we use chromatin immunoprecipitation with a DSB repair protein RAD51. To test crossover formation, we utilize a high-throughput pollen typing method, in which sperm cell nuclei are isolated using fluorescence-activated flow sorting and then genotyped with digital droplet PCR. Not all DSBs result in crossovers, depending on factors such as the chromatin state and the level of inter-parental DNA sequence polymorphism. In addition to potential practical applications of this research, we hope to shed light on factors affecting DSB fate.

Gene / Gene Models described: *SPO11-1*; GRMZM2G129913
Funding acknowledgement: Meiogenix company

P55

The maize E3 ligase ZmCER9 specifically targets activated NLRs for degradation

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Plant disease resistance proteins of the nucleotide binding leucine-rich repeat (NLR) type are activated upon recognition of specific pathogen-derived effector proteins and induce a strong defense response, known as effector-triggered immunity or ETI, which often includes a rapid localized cell death known as the hypersensitive cell death defense response (HR). The maize NLR protein Rp1-D21, an autoactive derivative of the Rp1-D common rust resistance protein, triggers a spontaneous HR in the absence of pathogen challenge. ZmCER9, a member of the E3 ligase family associated with endoplasmic reticulum-associated degradation (ERAD), emerges as a crucial player in this process. Through genome-wide association (GWA) mapping, we identified ZmCER9 as a candidate among the modifier loci influencing Rp1-D21-induced HR. Our study characterizes ZmCER9 as an active E3 ligase localized to the endoplasmic reticulum, where it facilitates the proteasome-dependent degradation of Rp1-D21. Intriguingly, ZmCER9 exhibits a broader spectrum of influence, suppressing and directing the degradation of autoactive NLRs from maize, Arabidopsis, and barley. Extending our investigation to Arabidopsis, we observe an increased hypersensitive response (HR) to specific pathogens in the absence of AtCER9, highlighting the conservation of the CER9-mediated degradation mechanism across plant species. In conclusion, our findings unveil a previously undiscovered process in plants involving ZmCER9 and the ERAD system, providing insights into the degradation of NLR proteins of immune homeostasis.

Gene / Gene Models described: *ZmCER9*; Zm00001eb387290
Funding acknowledgement: National Science Foundation (NSF)

P56 @HollyPlants

Transcriptomic variation during kernel development in near-isogenic purple maize cultivars

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Purple maize kernels are characterized by the accumulation of anthocyanins in the pericarp tissue. As water soluble pigments, anthocyanins are of interest to the food industry as natural food dyes. Anthocyanins possess health-promoting properties and are shown to reduce biomarkers associated with inflammation, diabetes, and adipogenesis (Zhang et al., 2019). To understand the genetics influencing anthocyanin accumulation during purple maize kernel development, we conducted an RNA-seq study on pericarp tissues collected from three near-isogenic purple maize cultivars and B73 at 10, 15, and 20 days after pollination (DAP). Differential gene expression (DGE) analysis identified significantly up- and down-regulated genes that are functionally characterized as involved in flavonoid biosynthetic and metabolic processes across developmental stages. For example, *Hct10* and a novel P450 gene model are significantly upregulated at 10 DAP. In an anthocyanin rich B73 isoline, 10 and 15 DAP were enriched in flavonoid, anthocyanin, and luteolin biosynthetic processes, while 20 DAP exhibited increased enrichment for phenylpropanoid processes. This study identifies metabolic loci associated with anthocyanin biosynthesis, as well as potential candidate genes. Future analysis of the data using variant calling software can pinpoint allelic variation for marker-assisted selection for increased anthocyanin concentration.

Gene / Gene Models described: *Hct10*; Zm00001eb366700, Zm00001eb351260

P57

Transformation of Fast Flowering Mini Maize (FFMM)

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Fast Flowering Mini Maize (FFMM) is a line that has a generation time under two months. It is being used worldwide for various studies, particularly those needing controlled environments, and by those who desire quick results. We are developing methods to use FFMM to expedite maize transformation and gene editing. FFMM has been transformed using BabyBoom (BBM) and Wuschel (WUS), but subsequently with only WUS2 to minimize the effect on plant morphology. Constructs have been assembled for the eventual stacking of multiple transgenes in FFMM with the ability to remove the selectable marker and WUS2. A landing site on a GFP marked B chromosome for insertion via recombination mediated by phiC31 recombinase has been introgressed into FFMM for multiple generations. Insertions into this B chromosome will permit extensive dosage analyses of the transformed genes. Gene editing within FFMM was demonstrated by mutating the nondisjunction factor gene on a phenotypically marked B chromosome introgressed into FFMM. A haploid inducer derivative of FFMM has been produced that has GFP and RUBY phenotypic markers that will aid in the identification of haploids. With the addition of editing constructs to this inducer, the ability to edit any target line via Haploid Induction-Edit with an expedited timeframe will be possible. Various approaches are being tried to reduce the transformation time as much as possible.

Funding acknowledgement: National Science Foundation (NSF)

P58

Unlocking meiotic recombination in the maize genome.

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Meiotic recombination involves the programmed exchange of DNA material between homologous chromosomes, known as crossing-over (CO). In maize, as in many other higher eukaryotes, only a small fraction of the genome (approximately 5%) harbors COs. Furthermore, CO sites are mostly clustered at defined genomic locations called CO hotspots. In maize mechanisms that maintain CO hotspots and establish new ones remain poorly understood. Our research indicates that decreased nucleosomes density and reduced DNA methylation are critical features of CO sites in maize. Thus, changing the chromatin state may impact CO landscape. To test this hypothesis in maize, we investigated the effects of chromatin structure changes on meiotic recombination using two maize mutants: *decreased DNA methylation* (*ddm1*) and *methyltransferase2* (*zmet2*). These studies demonstrated that mutations of the *Ddm1* and *Zmet2* genes resulted in an increased number of COs and reshuffled CO patterning chromosome-wide. The *ddm1* mutation increased CO numbers by 70% and lead to the formation of new CO hotspots at distal chromosome sites, while the *zmet2* mutation boosted COs by 22% and redirected them to interstitial chromosome segments. Interestingly, both mutants displayed COs at genomic sites where COs are not detected in wild type. Detailed analysis of the CO sites in the mutants revealed two separate mechanisms of unlocking genomic loci for recombination. COs *ddm1* exhibited reduced DNA methylation but no significant changes in nucleosome density, whereas COs in *zmet2* showed decreased nucleosome density without a substantial decrease in DNA methylation. These results demonstrate that altering in chromatin structure can unleash meiotic recombination in chromosome regions that are normally recombination inert, therefore possible will be to create recombination landscape by design.

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P59

A KNOX-BLH homeodomain complex targets a major domestication locus

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Flowering teosinte initiates several small female inflorescences in each leaf axil compared to domesticated maize that produces only a single large one. A major QTL controlling this phenotype, *prol1.1*, maps to a 2.7 Kb region upstream of the *grassy tillers1* (*gt1*) homeodomain leucine zipper gene known to play a role in repression of tillering and carpel abortion in tassels. Our previous work identified a potential KNOX homeodomain binding site at *pro1.1* that was confirmed via ChIP-sequencing using a KNOTTED1 (KN1) antibody. Because KNOX homeodomain proteins are known to dimerize with BLH homeobox proteins, we investigated whether a KNOX/BLH complex could bind to *prol1.1*. From transcription profiling we discovered that *gt1* transcripts are highly downregulated in *blh12/14* mutants that are defective in intermediate vein patterning, making the functionally redundant BLH12/14 proteins good candidates to be members of a complex. Furthermore, pull-down experiments indicated that KN1 and BLH12 or BLH14 proteins could physically interact as a complex in vivo. To verify potential binding at *prol1.1*, ChIP-seq using a BLH14 specific antibody on male and female inflorescence chromatin was done. This confirmed binding of BLH proteins to *prol1.1*, with a binding peak that overlaps completely with the KN1 peak. Interestingly, GT1 imunolocalization in *kn1* or *blh12/14* mutant backgrounds revealed reduced expression in axillary meristems, both vegetative and floral. This finding, together with the RNAseq results, indicate that the KNOX/BLH complex functions to activate *gt1* in axillary positions where it may function to repress growth. This activation, however, occurs within a window after axillary meristem formation, but before lateral organ initiation as a mechanism to prevent excess branching late in development.

Gene / Gene Models described: *BLH12, BLH14, KN1*; Zm00001eb147970, Zm00001eb289480, Zm00001eb055920
Funding acknowledgement: National Science Foundation (NSF), United States Department of Agriculture (USDA)

P60

A nuclear moonlighting function of maize trehalose-6-phosphate phosphatase in inflorescence branching

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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 phase separates and 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 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.

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P61

A regulatory module of LITTLE ZIPPER and ROLLED LEAF1/REVOLUTA controls ligule development in maize

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The ligule is a grass-specific leaf appendage that, along with the auricle, comprises the blade-sheath boundary. Ligule initiation from the adaxial epidermis of developing leaf primordia is the first morphological demarcation of the blade and sheath. This patterning event has become a classic developmental model for studies on cell fate diversification in grasses. However, gene regulatory networks that control the precise formation of this unique structure remain largely unknown. We identified the conserved LITTLE ZIPPER-ROLLED LEAF1/REVOLUTA (ZPR-RLD1/REV) module as a key regulator of maize ligule development. Disrupting three *ZPR* genes (*ZmZPR3*, *ZmZPR4a*, and *ZmZPR4b*) simultaneously caused defective ligule formation and upright leaf blades. Subsequent genetic analysis of single, double, and triple *Zmzpr* mutants revealed redundant functions within the ZPR family, highlighting their collective requirement for proper ligule formation. Arabidopsis ZPR proteins were previously shown to interact with REV, an HD-ZIPIII transcription factor that promotes ad/abaxial polarity during leaf development. Using yeast two-hybrid assays, we confirmed protein-protein interactions between all three ZmZPR proteins and RLD1/ZmREV1, an ortholog of Arabidopsis REV. Previously reported mis-expression of *RLD1/ZmREV1* in dominant *Rld1* mutants notably induces ectopic ligule development on the abaxial leaf surface at the blade-sheath boundary, underscoring its critical role in establishing the ligule. Additionally, we examined genetic interactions between triple *Zmzpr* mutants and *liguleless2* (*lg2*) and found that quadruple mutant leaves phenocopied *lg2*, suggesting that the *LG2* encoded bZIP domain TGA transcription factor may be a downstream target of the ZmZPRs in ligule development. This, along with overlapping mRNA expression patterns of *ZmZPRs*, *RLD1/ZmREV1,* and *LG2* in leaf primordia, highlights a diverse function of the ZPR-REV regulatory module in maize. Taken together, our findings unveil the ZPR-REV module as a novel regulator of ligule formation, potentially mediated through *LG2* regulation.

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P62

A spatial transcriptome map of the developing maize ear

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A comprehensive understanding of inflorescence development is crucial for crop genetic improvement, as inflorescence meristems give rise to reproductive organs and determine grain yield. However, dissecting inflorescence development at the cellular level has been challenging owing to a lack of specific marker genes to distinguish among cell types, particularly in different types of meristems that are vital for organ formation. In this study, we used spatial enhanced resolution omics-sequencing (Stereo-seq) to construct a precise spatial transcriptome map of the developing maize ear primordium, identifying twelve cell types, including the four newly cell types found mainly in the inflorescence meristem. By extracting the meristem components for detailed clustering, we identified three subtypes of meristem, and validated two MADS-box genes that were specifically expressed at the apex of determinate meristems and involved in stem cell determinacy. Furthermore, by integrating single-cell RNA transcriptomes, we identified a series of spatially-specific networks and hub genes that may provide new insights into the formation of different tissues. In summary, this study provides a valuable resource for studies of cereal inflorescence development studies, offering new clues for yield improvement.

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P63

Analysis of Pistachio root proteome to salt stress

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Pistachio (Pistacia vera L.) is an economically important tree nut that commonly thrives in semi-arid and arid environments. P. vera is a highly adaptable to various abiotic stresses, and it can tolerate drought and salinity stresses, which makes it suitable for reforestation of arid and salinized zones. However, the mechanisms underlying the salinity tolerance of this plant are not well understood. The present study was aimed at physiological and molecular investigations to unravel the metabolic pathways associated with the salt tolerance mechanisms in UCB-1 cultivar. Five one-year-old pistachio rootstocks were treated with four saline water regimes for 100 days. The rootstocks adopted Na+ exclusion strategy to resist the salinity stress. Total proteins were isolated from the roots and treated with different NaCl concentrations. The proteins were characterized using high throughput LC-MS/MS spectrometry searched against the Citrus database. Over 1600 protein IDs were detected, among which the comparative analysis revealed 245 more abundant and 190 low abundant proteins to three stress levels. The proteins associated with amino acid metabolism, cell wall organization, protein homeostasis, response to stress, signal transduction, TCA cycle, and vesicular trafficking were constantly overexpressed at all stress levels. At low and moderate stress levels, the chromatin and cytoskeleton organization lipid metabolism proteins were overexpressed, while at higher salt concentrations, they were unaffected. Transcription and translation processes were affected by all stress levels, as the proteins showed down-regulation in response to all stress levels. Transcription proteins were downregulated at low and moderate stress while overexpressed at high salt stress treatment. Protein interaction network with all the orthologous proteins mapped to Arabidopsis thaliana and the clusters associated with these proteins revealed the cytosolic, carbohydrate, and amino acid metabolism are associated with salinity stress.

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P64

Automated image analysis of maize pollen for phenotypic measurements

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The Fowler lab is interested in pollen development and how it relates to plant reproduction, particularly in maize. Previous work has shown that for at least one pollen mutant, stt1, reduced pollen grain size correlates with reduced pollen grain germination (Phillips, 2011). As such, it would be beneficial to investigate the variation and breadth of pollen grain size and shape phenotypes across maize lines, including specific mutants impacting pollen fitness, as well as inbred lines capturing the genetic diversity across the species. A single maize plant can produce several million pollen grains, all containing unique haploid genomes following meiosis, and such large populations could be utilized to generate rich phenotypic datasets. This project is developing the automation of large-scale pollen grain measurements. Key to this approach are the plating of pollen, the microscope utilized, automation of the imaging process, and the ability to selectively differentiate and measure objects in accordance with user-specified parameters. The current working model is adapted from established approaches used for measurement of microscopic features like nuclei (Schindelin et al, 2012). This approach has been validated with an established pollen grain size mutant (stt1). Preliminary results demonstrate that this approach can be used to detect differences in ploidy, or detection of aberrant pollen morphology. Additionally, we have identified subpopulations of differing size in single heterozygous populations (stt1, stt2, and stt3) as well as in double heterozygous populations (MDR1 / DNG102), which we are using to validate the robustness of this approach when applied to complex or mixed populations of pollen.

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P65

Catalytic and non-catalytic TREHALOSE-6-PHOSPHATE SYNTHASES (ZmTPSs) interact with RAMOSA3 to control embryo and post-embryo development

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Trehalose-6-phosphate (T6P) is the intermediate in the two-step pathway of trehalose biosynthesis mediated by T6P-synthase (TPS) and T6P-phosphatase (TPP). Plants harbor small families of *TPS* and *TPP* genes and most plant TPSs lack enzymatic activity, suggesting they might have regulatory functions. In maize, the classical mutant *ramosa3* (*ra3*) increases inflorescence branching and RA3 encodes a catalytic TPP. To further explore the molecular mechanism of RA3's functions, we screened for its interactors and found that RA3 interacts with ZmTPS1 and ZmTPS12, two non-catalytic TPSs. *zmtps1* and *zmtps12* mutants enhance *ra3* phenotypes, suggesting their interaction is biologically significant. Interestingly, we found that ZmTPS1 also interacts with the two catalytic active TPSs, ZmTPS11 and ZmTPS14. We knocked out these genes using CRISPR-Cas9, and *zmtps11;zmtps14* double mutants fail to complete embryogenesis, suggesting that the active TPSs are important for embryo development, as in Arabidopsis. Next, to ask if the TPS-TPP interactions affect enzyme activity, we performed a coupled enzyme assay, and found that the non-catalytic ZmTPS1 stimulated the coupled activity of RA3 and ZmTPS14. This result also suggested that RA3, ZmTPS1, and ZmTPS14 form a complex, and we confirmed it by expressing and purifying the three proteins from insect cells. Moving forward, we will visualize the complex by cryo-electron microscopy. This will allow first insights into the structural basis and stoichiometry of TPS-TPP interactions, which have not been studied in any organism.In summary, our results suggest that 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 also provided insights for the first time into the combined activity of the two major trehalose gene classes, TPSs and TPPs, in plant development.

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P66

Cause and consequences of abnormal Meiosis II division in *bige7* heterozygotes

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Big embryo7 (*Bige7*) encodes the BRIZ1 subunit of the universally conserved BRAP/BRIZ E3 ligase, implicated in animal cell cycle regulation and pleiotropic roles in plant development. The suite of *bige7* phenotypes includes defects in seed germination, aleurone differentiation, meiosis II, and male gametophyte development. Interestingly, the latter two phenotypes occur in heterozygotes. Reciprocal crosses confirmed normal transmission through the female. Analyses of pollen morphology and germination rates have thus far not established a basis for reduced transmission. However, abnormal tetrad shapes in *bige7*/+ anthers are attributed to one cell of the dyad mis-dividing while the other cell divides normally. Both this pattern, and pollen transmission ratio, are consistent with uncovering of recessive *bige7* in dyad cells by meiosis I segregation. To test the meiosis I segregation hypothesis, *bige7* was crossed with inbred Ki11, which carries a knob (K2S) that is linked to the *BigE7* locus, both on 2S. Dividing dyad meiocytes and tetrad microspores from F1 anthers were genotyped for knob constitution by FISH using a K-180 probe. Inferred dyad *bige7* genotypes were then correlated with meiosis II division angles measured by 3D rendering and image processing using ICY and ImageJ. Meiosis II division angles of homozygous recessive *bige7* dyad cells showed significantly larger deviations from normal division compared to heterozygous and wild type dyad cells, providing unambiguous support for the Meiosis I segregation hypothesis. The hypothesis has striking implications: 1) *BigE7* is transcribed in dyads during the brief interkinesis stage of meiosis, and 2) fates of *bige7* microspores depend on the genotype of their diploid dyad progenitor implying an unexpected connection between meiosis II division and microspore development. Because few essential genes are thought to be expressed during interkinesis, meiosis I segregation is assumed to have little impact on male fitness. In contrast, the *bige7* phenotype challenges that assumption.

Funding acknowledgement: National Science Foundation (NSF)

P67

Characterizing the phenotypic rescue of D-erythrose in salt-stressed maize and its role in stress-response regulation and development

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Increased drought due to climate change has exacerbated soil salinization, particularly in agricultural systems where irrigation practices already contribute to excessive salt accumulation. Salt stress is a major constraint on maize yield, and has been shown to stunt root and shoot growth and decrease lateral root count. In order to study the mechanisms of stress response and development in maize, we are investigating metabolites localized to the meristem region of maize roots exposed to salt stress. Since the meristem is the site of stem cell differentiation, metabolite localization to this region could indicate increased growth response during stress, revealing an uncharacterized role of metabolic stress signaling. To determine spatial localization of metabolites during stress, we are developing a new data analysis pipeline for mass spectrometry imaging that combines data collected from desorption electrospray ionization-mass spectrometry imaging (DESI-MSI), high-performance liquid chromatography-mass spectrometry (HPLC-MS), and computational analysis to determine and confirm chemical composition and spatial localization. These techniques identified the sugar D-erythrose, a compound that localizes to the meristem region of maize roots. We found that salt-stressed maize treated with D-erythrose conferred phenotypic rescue in root growth. Phosphorylated erythrose is a known intermediate in the Calvin cycle and shikimate pathway, which are important for energy and amino acid production. Current investigations show that D-erythrose treatment significantly rescues root growth compared to the same concentration of D-sucrose treatment, suggesting an additional effect beyond providing energy supply. Erythrose could have important applications in terms of stress response signaling or have other roles in plant development regulation. We are continuing to characterize the role of erythrose during salt stress using chemical and genetic approaches.

Funding acknowledgement: National Institutes of Health (NIH), SACNAS ROOT and SHOOT Travel Award

P68

Cloning the classic maize metaxylem mutant, wilted1 (wi1) by chromosome walking and RNA sequencing

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Plant growth is dependent on multicellular vascular bundles and their ability to conduct water. The majority of water transport in maize is accomplished by xylem vessel elements, protoxylem and metaxylem. Vessel element cells are excellent water conductors because they undergo Programmed Cell Death (PCD), a process that is regulated by NAC and MYB family genes. The classical maize mutant, wilted1 (wi1), exhibits a water transport defect linked to xylem vessel element production. Although protoxylem in wi1 develops normally, its metaxylem is delayed, resulting in a dwarfed and water deficient phenotype. Chromosome walking localized wi1 to a 82 Mbp interval on chromosome 6 including the centromere. Analysis of 20 F2 individuals showed that wi1 fits expectations for a single recessive allele. We characterized stem metaxylem in a 1:1 wi1:WT population and found that wi1 plants lack metaxylem in the lowest internodes at the V5 growth stage, before any obvious stress response. Using our early phenotyping method, we prepared RNA from 6 wi1 and 4 WT individuals for RNA sequencing and differential gene expression analysis. Our RNAseq identified 1174 differentially expressed genes (DEGs) between wi1 and WT, with 76% of DEGs upregulated in wi1, suggesting that WI1 is a transcriptional repressor. GO Term enrichment suggests that plant stress and transcriptional regulation are upregulated in wi1, while photosynthesis and cell expansion are downregulated in wi1. While we originally anticipated wi1 to be derived from a loss of function of a NAC or MYB within the mapping interval on chromosome 6, we instead find the most likely candidate gene to be a complex locus that contains Zm00001eb265940, Zm00001eb266010, and Zm00001eb266000, annotated as one alpha mannosidase - lipase pseudogene, LOC103630979 in the NCBI NAMv5.0 assembly. Ongoing work aims to confirm the structure of this transcript by RT-PCR and determine its protein coding potential.

Gene / Gene Models described: *wi1*; Zm00001eb265940, Zm00001eb266010, Zm00001eb266000
Funding acknowledgement: National Science Foundation (NSF), IOS-2211434, IOS-2211435

P69

Comparison of DNA replication timing profiles between B73 and NC350 maize lines

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Cells in the root tips of B73 and NC350 maize lines replicate their DNA in approximately 3 hours in a highly regulated, temporally ordered process. By using a multi-inbred approach, we aim to characterize the evolution and conservation of DNA replication in maize. We employed a flow cytometric and DNA copy number protocol to assign a replication time (RT) to every region of the maize genome. DNA copy number methods rely on precise, high quality mapping protocols. Therefore, we developed an in silico strategy to identify segments of maize reference genomes with low inherent "mappability", which we employed as drop lists during downstream genomic analysis. Using nuclei collected by flow sorting, we obtained a ratio of the read numbers at each locus for nuclei in S phase relative to those in G1 phase. Because loci replicating early in S phase will have higher copy numbers, this data can be used to assign a replication time to each locus. Comparison between NC350 and B73 replication programs is particularly interesting considering NC350's tropical lineage, larger genome size, and higher knob count than other NAM lines. Centromere and knob regions typically replicate later in S phase, which makes these genomic areas and the genes around them of special interest. While most genes replicate at a similar time in S phase in these two maize inbred lines, there are some notable differences. This project aims to uncover regions where the timing of DNA replication diverges between B73 and NC350 and to explore the genomic and genetic contexts that influence replication time.

Funding acknowledgement: National Science Foundation (NSF)

P70

Coupling computer vision with spatial analysis of cell patterning allows the genetic basis for stomatal density to be dissected into component traits related to known developmental mechanisms in maize

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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 reveal how stomatal patterning influences leaf gas exchange. However, the genetic architecture of stomatal patterning is primarily understood only for the coarse, composite traits 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 mosaic, or tessellation, of stomata on the maize leaf surface in terms of 2D kernel density distributions (KDDs). A set of traits describing variation in the KDDs was subjected to principle components analysis and structural equation modeling for dimension reduction and to assess trait interactions. The trait set includes features consistent with known developmental mechanisms regulating independent elements of patterning along the medial versus longitudinal axes of the leaf. Linear models featuring the core trait set explained 74% of the variation in SD. Ten quantitative trait loci (QTL) for SD also associated with KDD-derived patterning traits. Seven SD QTL were exclusively associated with medial KDD-derived traits. Three QTL were associated with both medial and longitudinal KDD-derived traits. Medial QTL also colocalized with specific leaf area QTL i.e. measures of leaf blade thickness and density. Overall, these spatial methods provide a case study for atomizing histology into orthogonal components, identifying their distinct genetic drivers, and refining our understanding of how complex traits like SD arise.

Funding acknowledgement: Department of Energy (DOE)

P71 @ClFGonzales

Crosstalk between auxin and jasmonic acid in maize root development

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Phytohormones play crucial roles in regulating plant growth, development, and environmental responses. Extensive studies in model plants such as *Arabidopsis* have demonstrated that plant hormones can have distinct, overlapping, and antagonistic functions. For example, both auxin and jasmonic acid (JA) can inhibit root growth and JA-auxin crosstalk has been well-studied in *Arabidopsis*. Root growth inhibition by JA and auxin in maize suggests that these hormones may also be integrated during maize root morphogenesis. To address this knowledge gap, we are employing hormone response assays, gene expression analyses, and in vivo quantification of hormone levels. An auxin signaling mutant, *arf27*, exhibited insensitivity to JA compared to wild-type W22 seedlings. JA biosynthesis and signaling genes are decreased in *arf27* compared to W22 based on RNA-seq data, which is in line the arf27 JA response phenotype. To determine if these transcriptional changes can impact hormone levels in vivo, hormone levels will be examined in W22 and *arf27* in the presence or absence of exogenous auxin treatment. The findings of this study will provide valuable insights into JA-auxin crosstalk during maize root development.

Gene / Gene Models described: *arf27*; GRMZM2G160005
Funding acknowledgement: United States Department of Agriculture (USDA)

P72

Defining the gene regulatory networks of *bHLH122* and *bHLH51* in maize anther development

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Maize tassels contain thousands of anthers, which produce the male gametophyte, pollen. Fully differentiated pre-meiotic 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 meiocyte through pollen maturation. To support meiocyte and pollen development tapetal cells become binucleate, secretory, and finally undergo programed cell death. A hierarchy of four basic helix-loop-helix (bHLH) transcription factors (TF), *Male Sterile(MS) 23*, *MS32*, *bHLH122*, and *bHLH51* regulate tapetal cell differentiation and development. Mutants in any one of these bHLH TFs results in complete male sterility due to a failure of the tapetal cell layer to fully differentiate. *Ms23*, *Ms32*, and *bhlh122* act upstream of and are required to produce 24-nt pre-meiotic phased secondary small interfering RNAs (phasiRNAs) and *bHLH51* integrates into this regulation hierarchy downstream of *24-PHAS loci* activation. Here we test if the male sterile phenotype of *bHLH122* and *bHLH51* CRISPR knock out alleles is complemented by epitope tagged copies of these genes. These lines will allow for future molecular work to define the gene regulatory networks of *bHLH122* and *bHLH51*.

Gene / Gene Models described: *Ms23, Ms32, bHLH122, bHLH51*; Zm00001eb332170, Zm00001eb106620, Zm00001eb251650, Zm00001eb208200
Funding acknowledgement: National Science Foundation (NSF)

P73

Determining the impact of plant architecture on lifespan stalk flexural stiffness

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Plant mechanics is influenced by external dynamic forces interacting with internal forces from developing architectures. However, the mechanisms by which plants adjust their mechanics to adapt to these forces is unknown. To quantify changes in biomechanics over lifespans, we measured stalk flexural stiffness in B73 and Mo17 plants from the vegetative tasseling (VT) stage until reproductive maturity (11 weeks total testing). Our results show that B73 plants are stronger than Mo17 plants over their lifespan. Stalk flexural stiffness is determined by the geometry and material properties of stalks. To account for changes in stalk geometry, we normalized the flexural stiffness by diameter weekly. B73 plants were still stronger than Mo17 plants after normalization. This suggests that the difference in lifespan stalk flexural stiffness between Mo17 and B73 plants may be driven by material properties. We hypothesized that changes in material properties result from the differences in developing plant architectures. To test this hypothesis, we altered plant architecture by either detasseling or replacing tassels with prosthetic tassels at the VT stage. Our results show that Mo17 plants with prosthetic tassels are stronger than Mo17 plants with intact tassels, but neither were different from Mo17 detasseled plants. In contrast, B73 plants with prosthetic tassels were not significantly different from B73 plants with intact tassels but both were stronger than B73 detasseled plants. The influence of architectural alterations is unchanged after normalizing by diameter, thus supporting our hypothesis that material properties are influenced by developing architectures. Together, these findings provide a basis for further investigating the interaction between plant architectures, material properties, and changes in stalk mechanics.

Funding acknowledgement: National Science Foundation (NSF)

P74

Development of a lineage tracing line for maize brace root developmental studies

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Brace roots, a type of adventitious root, contribute to root lodging resistance in many plants, including Zea mays. The main stages of brace root development (initiation, elongation, and maturation) are understood; however, it is unknown which nodal cells begin the development process. We developed a lineage tracing tool to address this unknown; lineage tracing marks a progenitor cell and enables its progeny to be tracked. Using Golden Gate restriction enzyme cloning, a two-construct lineage tracing system was developed. One construct carries a CRE recombinase fused to a Cyclin B1 dBox, which is expressed under a heat shock-inducible promoter and only functional in mitotically active cells. The other construct contains a ubiquitous promoter driving a floxed terminator and 3xYFP. In a cell containing both components, heat shock induces CRE-dBox expression. CRE then recognizes the loxP sites in the second component and excises the terminator between 3xYFP and the promoter. This allows 3xYFP to be expressed, permanently marking that cell and all of its progeny. These constructs are being transformed into maize, and the transformed lines will be used to determine which nodal cells give rise to brace root primordia. Ultimately, this work aims to elucidate the mechanisms behind the initiation of brace root development and investigate biomechanical resistance to root lodging. An understanding of these phenomena could aid the design of root lodging-resistant crops. Root lodging-resistant crops could efficiently increase yield on currently available agricultural land, providing food to meet the demands of the continuously growing human population while minimizing agricultural land use.

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P75

Development of an accessible and scalable maize pollen storage technology

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Seed production in Zea mays (maize) is challenged by the inherent short lifespan of maize pollen. We developed a pollen storage technology that preserves maize pollen viability for up to seven days that delivers seed set equivalency to fresh pollen. This technology is simple and scalable for use cases ranging from single plant pollinations to hectares of commercial hybrid seed production. The protocol is based on key discoveries around regulating the respiration rate of metabolically active pollen during storage, optimization of the storage environment using low-cost, globally available materials, and furthered understanding of how small particles may interfere with pollen-silk interactions. Prior efforts in maize pollen storage focused on pollen conditioning and cryogenic preservation at the expense of technology scalability. We have broken down these accessibility barriers and increased protocol repeatability through an approach that places freshly collected pollen directly in a refrigerated storage environment where pollen is free to maintain cellular respiration using atmospheric air. Historical maize pollen storage technologies have prescribed foliated mineral powders or fluffy, amorphous silicates as anti-clumping agents to prevent pollens from sticking together during storage. Scanning electron microscopy and aniline blue staining suggests that this historical precedent was a key factor limiting the pollination performance of maize pollen through inhibition of pollen-silk interaction. This led to the discovery that crystalline materials with low specific surface area and the optimal range of particle sizes deliver a step change in seed set that can be achieved with stored pollen. This technology is to be published for the greater benefit the global maize biology and production community.

P76 @kswentowsky

Developmental genetics and genomics of perennial regrowth in *Zea diploperennis*

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Perennial plants regrow for multiple years and perennial crops are sustainable agriculture systems. However, breeding perennial maize has been largely unsuccessful due to our poor understanding of genes and developmental mechanisms that control perenniality. 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. By analyzing individuals that displayed regrowth at early and late time points in the perennial life cycle, we determined that *Reg1* affects initial regrowth while *Reg3* is necessary for multiple sustained cycles of perennial regrowth. 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. This will help us determine which developmental pathways are differentially regulated between annuals and perennials, and may reveal the genes that underly *Reg1* and *Reg3*. We also plan to study the developmental mechanisms that dictate perennial regrowth in *Z. diploperennis,* and how they differ between perennial and annual grass species. We hypothesize that: (1) the maintenance of *Z. diploperennis* tiller buds in a juvenile and non-flowering state is necessary for perennial regrowth; and (2) dormant tiller buds become reactivated through phytohormone or carbohydrate signaling after flowering to begin the next cycle of perennial growth. Understanding the developmental genetics of perenniality could aid in breeding of more sustainable crops.

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P77

Drawing the line: interactions of maize transcription factors to coordinate proximodistal and mediolateral patterning in the maize leaf

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The blade-sheath boundary (BSB) of the maize leaf comprises two distinct tissues, an epidermally-derived ligule and a hinge-like auricle, that demarcate the distal blade and proximal sheath identities. The recessive mutation *liguleless1*(*lg1*) deletes the ligule and auricle and thus provides a useful genetic tool for identification of genetic networks controlling BSB development. *WOX3* family double mutants *narrow sheath1; narrow sheath2* (*ns1/2*) delete the marginal domain leaving blunt leaf edges, whereas higher order *WOX3* triple mutants (*ns1 ns2 wox3a*) delete the lateral and marginal domains and cause severe ligule/auricle defects. Class I *KNOX* family genes function antagonistically with *WOX3* function during recruitment of leaf founder cells, and the *KNOX* gene *LIGULELESS3* is expressed early in BSB development. Previous transcriptomic analyses of initiating ligules identified overlapping expression patterns for *LG1*, *WOX3* family genes, and class I *KNOX* genes in the incipient BSB. In addition, *lg1* mutants form ectopic sheath margin tissue that extends into the blade asymmetrically, implicating interactive *LG1*, *WOX3,* and *KNOX* function during proximodistal and mediolateral patterning. Although transcriptional analyses have been conducted on initiating maize ligules, the genetic mechanisms of later-staged BSB development are unknown. Here, we utilize single-cell transcriptomics to examine multiple stages of ligule/auricle outgrowth and patterning. Additionally, *lg1 ns1 ns2* triple mutants condition surprising and synergistic phenotypes. *In situ* hybridizations support a model wherein the coordination of interactive *LG1*, *WOX3*, and class I *KNOX*es gene functions patterns robust proximodistal and mediolateral development. Previous attempts to generate maize WOX3 and LG1 fluorescent reporter-protein (FP) lines were unsuccessful, likely owing to complexities in maize promotor structures. Recently, mutants and FP lines of *LG1*, *NS1/2*, and *WOX3a* orthologous genes were generated in the grass model *Brachypodium distachyon,* to investigate the dynamic interactions of these transcription factors during proximodistal and mediolateral leaf development.

Gene / Gene Models described: *LG1, NS1, NS2, WOX3A, LG3*; GRMZM2G036297, GRMZM2G069028, Zm00001eb197430, GRMZM2G122537, GRMZM2G087741
Funding acknowledgement: National Science Foundation (NSF)

P78

Elucidating the role of *TERMINAL EAR1 (TE1)* in maize development and stress

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Maize *TERMINAL EAR 1* (*TE1*) encodes a predicted RNA-binding protein containing three conserved RNA recognition motifs. Loss-of-function mutants have pleiotropic developmental defects, including faster leaf initiation, altered phyllotaxy, dwarf stature, and a feminized tassel. Despite controlling various aspects of maize development, the mechanism of *TE1* action is poorly understood. RNA-immunoprecipitation and sequencing (RIP-Seq) using TE1-Yellow fluorescent protein (YFP)-expressing maize ear primordia revealed TE1 binding to mRNAs of multiple maize developmental genes, including *SQUAMOSA PROMOTER BINDING PROTEIN-LIKE* genes *TASSELSHEATH4*, *UNBRANCHED2*, and *UNBRANCHED3*. However, mRNA-Seq analysis of *te1* mutants did not identify a significant change in the levels of most candidate TE1-bound target mRNAs. In contrast, the proteins encoded by candidate TE1-bound mRNAs were more abundant in *te1* mutants, suggesting *TE1* may control its targets at a post-transcriptional level. We also identified TE1-YFP interacting proteins using proteomics, and Gene Ontology analysis revealed enrichment in stress granule assembly and regulation of translation categories. Consistent with this, a functional TE1-YFP fusion localized to cytoplasmic puncta. These puncta accumulate and enlarge following heat shock and co-localize with a stress granule marker. To ask if *TE1* functions in heat stress responses, we tested germination following heat treatments, and found that *te1* mutants were more sensitive to heat stress. Our results suggest that TE1 binding to developmental mRNAs and sequestering them in cytoplasmic granules may be part of a mechanism to negatively regulate plant development. Thus, we hypothesize that TE1 may function to control the important tradeoff between plant growth and stress responses. Further understanding of TE1 function could provide new insights to enhance sustainable food production and ensure food security in the face of a changing climate.

Gene / Gene Models described: *te1*; Zm00001eb144140
Funding acknowledgement: National Science Foundation (NSF)

P79

Engineering dominant maize yield-component improvement with gene editing

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Based on DNA nucleases, the first generation of CRISPR gene-editing tools create cuts in DNA and mutations from error-prone DNA repair. The resulting insertions or deletions most commonly lead to a recessive loss of gene function through a translation frame shift, disrupting a protein domain, or decreasing gene expression level. These types of mutations are typically recessive and will continue to be valuable to geneticists for assigning gene function. However, there are serious technical difficulties deploying recessive mutations for commercial crop improvement. Most genes are part of gene families with some degree of functional redundancy. Genetic redundancy is an even greater issue in polyploid genomes which are common in crop species. Because clonal crops like fruit trees and berries do not breed true and cannot be reproduced through self pollination without major changes in desired agronomic traits, they pose a particular challenge when using recessive gene edits as deployment would require efficient simultaneous editing of multiple gene copies in the clonal plant. Finally, an increasing number of crops, following from maize, are grown as F1 hybrids because of improved yield, uniformity, and seed IP protection. Engineering recessive loss-of-function in hybrid crops necessitates introduction of the gene edit into both hybrid parents which adds considerable cost, complexity, and regulatory hurdles. The difficulties with deploying recessive loss-of-function editing strategies in crops necessitates a different genetic approach, engineered genetic dominance. Unfortunately, examples of dominant mutations are relatively rare in mutant collections, despite dominant mutations being integral to plant evolutionary transitions and agricultural innovation. Here we show methods of improving maize yield component traits with genetically dominant gene edits. Specifically, we show that dominance can be deployed with large chromosomal rearrangements, targeted domain deletion, gene inversion, and releasing mechanisms of transcriptional and translational inhibition.

P80

Fast Turnaround Maize Transformation Service by Crop Bioengineering Laboratory at Iowa State University

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To meet the demand for plant transformation services in the research community, the Crop Bioengineering Laboratory (CBL) at Iowa State University was established in 2022 with a mission to conduct research and provide expertise and services in crop genetic transformation. CBL has been providing fast turnaround and highly efficient transformation service for maize inbred B104 to customers since then and received excellent feedback from our customers. Utilizing an improved *Agrobacterium*-mediated transformation protocol (Kang et al., 2022, Front Plant Sci. 13:860971. doi: 10.3389/fpls.2022.860971) along with enhanced vector and plant selection systems (Lee et al., 2023, Plant Physiol. 192:2598–2603. doi:10.1093/plphys/kiad231), CBL is able to complete a maize transformation project in 50 days from the date of initiation to the date of transplanting transgenic plantlets to the soil. The average transformation frequency is over 20%. CBL looks forward to providing more researchers with quality products and superior services at competitive prices. Please contact Dr. Jessica Ji (jjessica@iastate.edu) for more information.

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P81

Functional and genetic analysis of alternatively spliced transcript isoforms of a conserved RNA Binding Motif Protein 48 (RBM48) essential for the splicing of U12-type introns

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Accurate recognition and removal of introns from precursor mRNA by spliceosome is a fundamental but complex process essential for the expression of eukaryotic genes. In addition to the major group, most eukaryotic organisms contain a rare minor group of introns called U12-type. Minor introns constitute less than 0.5% of all introns and are spliced by a separate minor spliceosome. The parallel existence of two groups of introns and their biological relevance is unclear; however, mutations that disrupt the splicing of U12-type introns cause developmental defects in plants and animals. Our laboratory recently reported a conserved RNA Binding Motif Protein 48 (RBM48) gene required for splicing U12-type introns and encoding a core minor spliceosomal protein. A maize rbm48 mutant displays genome-wide disruption of primarily U12-type introns with a severe defect in kernel development. Intriguingly, the maize and human RBM48 encode several transcript isoforms by alternative splicing; however, the biological function of transcript isoforms in U12 splicing is not apparent. In this report, we have developed a genetic assay by CRISPR-Cas9-mediated knockout of the RBM48 gene in the human cancer-derived cell line K562 to investigate the biological function of RBM transcript isoforms. Using RT-PCR with primers flanking the U12-type introns, we demonstrate they are not efficiently spliced in RBM48 knockout (KO) K562 cells. We also show that by separately transfecting the wild type and three different alternatively spliced transcript isoforms of RBM48 in the mutant K562 cells, the splicing of U12-type introns can be restored or genetically complemented. This provides evidence for the important role of RBM48 transcript isoforms in U12 splicing. The data generated from these studies will be presented.

Funding acknowledgement: National Science Foundation (NSF), Research Excellence Fund Oakland University

P82

Functional characterization of maize nitrogen transporters

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Root systems perform multiple functions for plants, including anchorage and nutrient uptake. Past work has studied the trade-off between brace root mechanics and nitrogen uptake and it was found that bigger brace roots take up more nitrogen. Previous unpublished research also found that aerial brace and soil brace roots take up different amounts of nitrogen. It is hypothesized that this anomaly is caused by the differential expression of nitrogen transporters in brace roots. To test this hypothesis, RNA from previously collected brace root samples from five different genotypes was used to perform RT-qPCR, using primers for seven different nitrogen transporters, to determine how much of each nitrogen transporter is expressed in each brace root. Preliminary results show that some genes are differentially expressed between aerial brace roots and terrestrial brace roots. Additionally, five nitrogen transporter maize mutants were grown and phenotyped. Nitrogen uptake assays were performed on the aerial and soil brace roots to determine if the mutation affects the plant's ability to take up nitrogen and the plant's preference for nitrogen type. Bend tests were also performed on aerial and soil brace roots for each plant, and additional samples were taken for RT-qPCR. Mechanical data from the bend tests will be compared to the nitrogen uptake data to further understand the tradeoff, or lack thereof, between brace root mechanics and nitrogen uptake.

Gene / Gene Models described: ; AC205608.4_FG004, Zm00001d026695, Zm00001d004621, Zm00001d002962, Zm00001d038412, Zm00001d025894, Zm00001d063910
Funding acknowledgement: National Science Foundation (NSF), Royal Society

P83 @Yan_Geneticist

Genetic regulation of self-organizing azimuthal canopy orientations and their impacts on light interception in maize

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The efficiency of solar radiation interception contributes to the photosynthetic efficiency of crop plants. Light interception is a function of canopy architecture, including, plant density, leaf number, length, width, and angle, as well as azimuthal canopy orientation. We report on the ability of some maize genotypes to alter the orientations of their leaves during development in coordination with adjacent plants. Although the upper canopies of these genotypes retain the typical alternate-distichous phyllotaxy of maize, their leaves grow parallel to those of adjacent plants. A genome-wide association study (GWAS) conducted on this parallel canopy trait identified candidate genes, many of which are associated with shade avoidance syndrome (SAS), including *phytochromeC2* (*phyC2*). GWAS conducted on the fraction of photosynthetically active radiation (PAR) intercepted by canopies also identified multiple candidate genes, including *liguleless1* (*lg1*), previously defined by its role in ligule development. Under high plant densities mutant of SAS and *liguleless* genes (*lg1*, *lg2* and *Lg3)* exhibit altered canopy patterns, viz., the numbers of interrow leaves are greatly reduced as compared to non-mutant controls, resulting in dramatically decreased PAR interception. In at least the case of *lg2*, this phenotype is not a consequence of abnormal ligule development. Instead, *liguleless* gene functions are required for normal light responses, including azimuth canopy re-orientation.

Gene / Gene Models described: *lg1, lg2, lg3, phyC2*; GRMZM2G036297, GRMZM2G060216, GRMZM2G087741, GRMZM2G129889

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P84

Growth hormones BR and GA modulate spikelet meristem identity through the *RAMOSA1* pathway in Setaria and maize

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In grasses, inflorescence architecture impacts grain yield and harvestability. The degree of inflorescence branching and position of grain and pollen-bearing spikelets are determined by timing of spikelet meristem (SM) identity, where an indeterminate branch meristem acquires a determinate fate to initiate spikelet development. In maize, mutants impaired in axillary meristem determinacy or identity, e.g., *ramosa* mutants, have increased branching in tassels and ears, yet the underlying molecular mechanisms remain elusive.Here, we use model grass *Setaria viridis* to dissect the mechanisms underlying SM identity and determinacy. Setaria's unique inflorescence morphology offers an ideal system for this study; axillary branches terminate either in a spikelet or sterile bristle, the latter due to an imposed fate overriding SM identity. Using a mutagenesis screen, we isolated a spectrum of meristem identity mutants. Among these, *bristleless1* (*bsl1*) and *spikeletless* (*spkl*) displayed homeotic conversions from bristles to spikelets or spikelets to bristles, respectively. Genetic mapping indicated that brassinosteroids (BRs) and gibberellic acid (GA) were intimately involved in the spikelet vs bristle fate decision, and treatment with exogenous GA or BR inhibitor produced inflorescences with all bristles or spikelets, respectively. *bsl1* is defective in the ortholog of rice *D11*, a rate-limiting enzyme in BR biosynthesis, and we validated *spkl* as an ortholog of maize *ramosa1 (ra1)*. Genetic analyses in Setaria indicate that *bsl1* is epistatic to *spkl* and we propose a model where SPKL/RA1 regulates SM identity genes through interfacing with BR and GA pathways. We also isolated a mutant in maize *d11* (*Zmd11),* which had semi-dwarf stature and smaller tassels with fewer branches. Crossing *Zmd11* to the *ra1-R* allele suggested more complex genetic interactions underlying inflorescence phenotypes in maize. Our results highlight conservation and divergence of pathways governing SM fate between Setaria and maize, knowledge that can be harnessed for development of higher yielding cereals.

Funding acknowledgement: National Science Foundation (NSF)

P85

Heat stress at the bicellular stage inhibits sperm cell development and their transport into pollen tubes

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For a successful double fertilization process in flowering plants (angiosperms), pollen tubes each deliver two non-motile sperm cells towards female gametes (egg and central cell, respectively). Heatwaves especially during the reproduction period are threatening male gametophyte (pollen) development, which results in severe yield losses. By using maize as a crop and grass model system, we found strong seed set reduction when moderate heat stress was applied for two days during the uni- and bicellular stages of pollen development. We show that heat stress accelerates pollen development and impairs pollen germination capabilities, when applied at the unicellular stage. Heat stress at the bicellular stage impairs sperm cell development and their transport into pollen tubes. To understand the course of the latter defects, we used marker lines and analyzed the transcriptomes of isolated sperm cells. While heat stress also affects the expression of genes involved in transcription, RNA processing and translation, especially genes in DNA replication and the cell cycle were mis-regulated. This includes centromeric histone CENH3 and α-tubulin. Most mis-regulated genes are involved in transition from metaphase to anaphase during pollen mitosis II (PM II). Heat stress activates spindle assembly check point and meta- to anaphase transition genes in sperm cells. In summary, mis-regulation of the identified genes during heat stress at the bicellular stage explains sperm cell development and transport defects ultimately leading to sterility.

Funding acknowledgement: United States Department of Agriculture (USDA)

P86 @ThomasDresselh

Heat stress induced pollen tube growth arrest and sterility supported by a MaizeStressDB

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The reproductive phase in flowering plants is highly sensitive to ambient temperature stresses, with even a single hot day sometimes being fatal to reproductive success. Many studies of heat stress on crop plants have shown that pollen development and fertilization belong to the most sensitive reproductive stages. To study the sensitivity and contribution of the elongated stigma tissue of maize (silks) to reproduction under heat stress, we applied moderate heat stress before pollination and during pollen tube growth. We will show that moderate heat stress causes late growth arrest of pollen tubes ultimately leading to sterility. We further show that heat stress causes elevated levels of reactive oxygen species (ROS), which can be reduced by scavengers partly restoring pollen tube growth defects. Heat stressed silks show that among others hydrogen peroxide catabolic processes and bHLH transcription factor genes are downregulated, while NAC and other transcription factor genes are strongly upregulated. To further push the development of stress resistant/tolerant maize and to select genes for functional studies, we generated a transcriptomic and proteomic atlas of maize at various environmental conditions and over time. We applied three major abiotic stresses (cold, heat and drought) to seedlings over a period of ten days and generated a database and web interface named MaizeStressDB. Functional differentially expressed genes and proteins (DEGs and DEPs) as well as gene regulatory networks (GRNs) can be identified in the web browser and associated with core regulators in response to cold, heat and drought stress. The MaizeStressDB platform will now help to drive the development of maize varieties better adapted to transient heat stress during the reproductive phase.

Funding acknowledgement: German Research Foundation (DFG), Bavarian State Ministry for the Environment and Consumer Protection

P87

Herbicide safener Metcamifen significantly improves the recovery of doubled haploid maize seedlings via *in vitro* culture

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The production of doubled haploid plants is a valuable process in the toolbox of the modern plant breeder; however, chromosomal doubling achieved via contact with doubling agents e.g., colchicine, is highly stressful and often lethal to young plants. We sought to test whether herbicide safener compounds could improve the survival rate of *in vitro* culture maize embryos when contacted with the doubling agent colchicine. In our method, we supplemented the doubling media with the herbicide safener, Metcamifen, at varying concentrations from 200 g/L- 800 g/L. We extracted maize embryos from a variety of lines and advanced the putative haploid embryos to germination media. The healthy seedlings were then transferred to soil, grown to maturity, and self-pollinated under optimal glasshouse conditions. We report a significant increase in the survival rate of putative haploid maize embryos following our optimized protocol, and a significant increase in the return rate of doubled haploid seed relative to the control. This innovation substantially increases the efficiency of obtaining sufficient numbers of doubled haploid plants and thus enables breeders to better leverage the power of doubled haploid plants in their breeding programs.

P88

High-throughput root phenotyping to investigate phenotypic relationships between early auxin response and adult crown root phenotyping

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Maize root systems exhibit plastic phenotypes that are constrained by genotype. An outstanding question in the field is whether juvenile traits under controlled environments, such as hormone responses, can predict mature crown root phenotypes under field conditions. Previously, the Kelley lab has studied the response of 10-day-old maize seedlings to exogenous auxin in 617 genotypes from the Wisconsin Diversity panel. While reference genotypes such as B73 and W22 exhibit primary root inhibition following auxin treatment, numerous genotypes were auxin hypersensitive or insensitive. These ~20 genotypes were grown in the field and subsequently phenotyped to identify any adult crown root traits that could be indicative of the genotype's early auxin response. Some of the features were examined include root angle, surface area, and average root length. Manual phenotyping can lead to errors and bias, so a digital pipeline was designed using ImageJ, Root painter, and Rhizovision. Statistical analysis was performed across the genotypes to explore relationships between different phenotypic traits relative to B73. As analysis continues, we hope to find traits that are indicative of auxin response. In turn we also hope to predict certain adult phenotypes through the seedling auxin response.

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

P89

Individual and collective tissue-specific roles of BRASSINOSTEROID INSENSITIVE1 (BRI1) receptor-like kinase to plant development and BR signaling in maize.

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Plant architecture is a key determinant of grain yield in maize. Among the major plant growth regulators, brassinosteroids (BRs) affect multiple plant architecture traits, including organ size, sex determination, and leaf angle. However, its mechanisms remain poorly understood. We have characterized several BR biosynthesis and signaling mutants that emphasize the role of BRs in plant growth and development. We generated a mutant *brassinosteroid deficient semi-dwarf* (*bds1*) as a nonsense mutation in BR biosynthetic putative gene. We also identified the close homolog *bds2* and generated several mutant alleles by *Ds* transposon remobilization. Unlike *bds1* mutants, *bds2* single mutants are indistinguishable from wild-type plants. However, *bds1-R*;*bds2-Ds* double mutants exhibited enhanced developmental defects such as dwarfing and tassel feminization. We have also identified and characterized mutants of *cabbage* (*cbg1* and *cbg2*) BR biosynthetic genes from UFMu lines. Neither single mutant displayed obvious defects. However, *cbg1*;*cbg2* double mutants had extreme dwarf stature, smaller leaves, fewer tassel branches and reduced root growth. Thus, we hypothesize that these genes redundantly function to regulate plant development. We also investigated roles of *brassinosteroid insensitive1* (*bri1*) genes, which encode a receptor-like kinase serving as the BR receptor. Maize encodes five BRI1 members: a specific duplication of BRI1 (ZmBRI1a and ZmBRI1b), and three additional homologs (BRL1, BRL2, and BRL3*)*. To understand the tissue-specific functions of the individual receptors and their contributions to BR signaling, we have identified *Mutator* or *Ac/Ds* transposon mutants for all genes and stacked various receptor mutants to examine the 32 single, double, triple, quadruple, and quintuple mutants. In seedling shoots and roots we observed developmental defects across different genotypes. No genotypes with a functional *bri1a* or *bri1b* showed obvious growth defects. However, *bri1a*;*bri1b* double mutants exhibited extreme dwarfing and reduced root size, indicating functional redundancy. Plant growth defects were enhanced in specific higher order mutant combinations such as *bri1a;bri1b;brl1* triples and *bri1a;bri1b;brl1;brl3* quadruples, implying some tissue specificity.

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P90

Investigating links between pectin dynamics and meristem activity in maize

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Plant meristems serve as a center for growth; they house stem cells that both self-renew and provide cells for organogenesis. All plant cells, including meristematic cells, are surrounded by rigid cell walls, a complex polysaccharide matrix that must be remodeled when cells expand and divide. Most plants have Type I cell walls, which have relatively high levels of pectin. In contrast, maize and other grasses have Type II cell walls, with significantly lower levels of pectin and a greater abundance and variety of hemicelluloses. Despite the importance of meristems and the cell wall in regulating plant growth, relatively little is known about how the cell wall is integrated with meristem function, although pectin is emerging as a key component in Type I plant cell walls. For example, in Arabidopsis, de-methylesterification of pectin is required to increase cell wall extensibility and promote primordia outgrowth. The role of pectin in plants with Type II cell walls, including maize, is much less clear. Maize contains multiple meristem types that vary in determinacy, phyllotaxy and primordia identity, and thus is an excellent system to investigate the links between the cell wall and meristem function. We first investigated the role of pectin in maize floral meristems using three monoclonal antibodies that recognize specific pectic epitopes. Interestingly, pectin accumulates differently in the upper and lower florets. In the upper floret, de-methylesterified pectin accumulated in initiating organ primordia, similar to Arabidopsis, whereas methylesterified pectin accumulated on the apical surface of the meristem. In contrast, little staining for either epitope was observed in the lower floret. We also examined the localization of a specific epitope of another pectin, rhamnogalacturonan-I, which interestingly, accumulated specifically at the boundary between the upper and lower floret. To further understand the role of pectin in meristems, we are examining the localization of these three pectic epitopes in other inflorescence meristem types, in vegetative shoot meristems, and in developmental mutants that alter meristem activity. Indeed, pectin accumulation is altered in *bearded-ear* mutants, which have defects in floral meristem determinacy and identity. Our results suggest that despite its low abundance in Type II cell walls, pectin is remarkably dynamic during maize development and may contribute to meristem growth dynamics.

P91

Investigating the effect of environmental stress on metabolite localization and signaling in plants

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Abiotic environmental stress caused by climate change is threatening agricultural systems by inhibiting plant growth and development. Metabolite signaling regulates both stress response and development in plants, however, critical metabolite-driven links between development and stress response remain an open area of exploration. To investigate the link between development and stress response, longitudinal sections of maize roots were imaged using desorption electrospray ionization mass spectroscopy imaging (DESI-MSI) and matrix-assisted laser desorption ionization (MALDI)-MSI. These high-resolution imaging techniques enable visualization of the localization of stress-induced metabolites across the developmental gradient in roots. Many of the metabolites that we have identified are uncharacterized, we are using high-performance liquid chromatography (HPLC)-MS/MS to determine the chemical composition of these small molecules. We have also identified several known meristem-localized metabolites, including succinate, with differential localization in response to stress. Exogenous treatments with these metabolites show significant growth phenotypes during stress. The spatial identification of stress-responsive metabolites with MSI will reveal candidate metabolites that bridge stress response and development. Characterizing the biological effect of these metabolites, through a combination of chemical and genetic screens, will provide insights into the mechanisms that plants utilize to address abiotic stress in an effort to generate more stress-tolerant crop lines.

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P92

Investigating the role of higher-WOX3 transcription factors during robust patterning of leaf-margin orientation and tassel spikelet development

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In maize leaves, the distal blade projects away from the plant whereas the proximal sheath surrounds and supports the underlying stem. The orientation of the inner and outer edges of the overlapping sheath margins show robust, non-random patterning. Specifically, the outermost edge wraps around the innermost edge to form consistent, left margin/right margin "wrapper-tucker" pattern (W-T) persistent across plastochron stages. *In situ hybridizations* of the boundary gene *CUP-SHAPED COTYLEDON2* (*CUC2*) reveal asymmetric transcript accumulation in the wrapper and tucker edges of the emerging sheath primordial margins, suggesting a potential role for CUC2 in the establishment and maintenance of the W-T pattern. In specific combinations of higher-order mutations in *WUSCHEL-LIKE HOMEOBOX3* (*WOX3*) genes, W-T patterning is random and robustness is lost. To understand the molecular mechanisms underlying W-T patterning in grass leaves, we are conducting laser microdissection RNA-seq (LM-RNAseq) analyses of the marginal domains of emerging P4 leaf sheath primordia. Other higher-order mutations in *WOX3* genes condition defects in tassel spikelet morphology, particularly influencing the bract-like glumes. In the "*very narrow sheath (vns; i.e. ns1 ns2 WOX3A+/wox3a")* mutant, glumes are significantly narrowed, creating an open spikelet that exposes the stamens before anthesis. Conversely, *wox3* triple mutants (*ns1 ns2 wox3a*) displays distinctively forked glumes that split at the end. Both the *vns* and *wox3* triple mutant phenotypes demonstrate a tendency toward feminization, notably evident in spikelets at the distal tip of the primary tassel branch. LM-RNAseq and spatial transcriptomic analyses will be used to elucidate the developmental mechanisms of WOX3 function in maize leaf and floral development.

Gene / Gene Models described: *CUC2, WOX3A, NS1, NS2*; GRMZM2G393433, GRMZM2G122537, GRMZM2G069028, Zm00001d052598
Funding acknowledgement: National Science Foundation (NSF)

P93

Is more always better? Progress and promise of autotetraploid sweet sorghum

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Increasing ploidy levels in plant species has been shown to increase cell size. The benefits of increasing cell size have been used in crops such as potato and sugarcane to produce larger yields. In maize, increasing ploidy results in increased grain size but not yield, which has limited its usefulness. However, increasing ploidy can potentially be employed for bioenergy crops where seed yield is not the primary target. Current efforts to produce ethanol from sorghum stem sugars for the Sustainable Aviation Fuel (SAF) market are not economically feasible, driving the need to increase sorghum's sugar production. A potential solution is to increase the ploidy level in sorghum to create bigger cells that can store more juice and sugar in the stem. A colchicine-induced autotetraploid sorghum and its progeny were validated using flow cytometry. Diploid and M_2 generation autotetraploid plants were grown in both the field and greenhouse for characterization. Autotetraploid plants had stomata that were 1.5x larger than diploids, leading to differences in stomatal density. Leaf gas exchange was measured using a Li-6800 to assess differences in net CO_2 assimilation, stomatal conductance, and transpiration efficiency. No significant differences between ploidy levels were observed for any gas exchange parameters. In field grown trials, both diploid and autotetraploid sorghum were juiced to measure biomass, sugar concentration (brix), and juice volume to determine sugar storing properties. Total biomass, juice volume, and brix were all significantly higher in diploids, while percent sugar per dry weight and total sugars were not significantly different. The lower values observed in the autotetraploid were not unexpected given previous reports on the performance of inbred autotetraploid lines. We hypothesize that generating double-cross hybrid autotetraploid lines will result in increased vigor through progressive heterosis. Currently, autotetraploid sorghum hybrids are being generated to explore the potential of a double-cross hybrid sorghum.

Funding acknowledgement: Department of Energy (DOE)

P94

K-means clustering analysis of gene expression across endosperm development

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Approximately 90 million acres are devoted to the cultivation of maize in the United States. which is crucial for sustaining various industries. This process, integral for a stable supply of essential products, emphasizes the need for Cresearch to enhance each stage of maize cultivation. A key aspect involves understanding the role of zeins in maize development; during endosperm maturation, an increase in zein production provides nutrition for the embryo during germination. Our goal is to classify and comprehend gene expression patterns in maize endosperm, shedding light on the intricate processes regulating its development.
K-means clustering is used to organize data into groups/clusters based on similarities. By using K-means clustering on maize endosperm expression data, we can reveal various expression patterns over time and uncover the molecular events that coordinate endosperm development.The objective of this study is to identify clusters of co-expressed genes, aiming to uncover patterns present during the crucial stages of maize endosperm development. For example, we may observe potential gene regulators based on a rise in expression just before the gene(s) of interest become more highly expressed. Initial results revealed patterns of gene expression during development. We identified groups with timepoint-specific expression patterns with peak expression occurring at early, middle, and late points in endosperm development. Other clusters showed groups of genes that were expressed consistently throughout development. These findings may pave a path for understanding molecular mechanisms underlying endosperm development and offer potential targets for further investigation.
Moving forward, this project is part of a broader time point analysis of endosperm expression. This research aims to contribute insights into gene regulatory networks during the intricate process of endosperm development to explore gene expression patterns across various time points in reciprocal crosses of two maize cultivars.

Funding acknowledgement: National Science Foundation (NSF)

P95

KATANIN's role in cell division positioning and cell elongation in maize

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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. The p60 catalytic subunit forms hexamers and is sufficient to sever microtubules through ATP hydrolysis. However, binding of the protein complex to microtubule severing sites is mediated by the p80 subunit, allowing for higher severing efficiency of microtubules. In Zea mays (maize), several katanin (p60) mutants have been identified, including a loss-of-function double mutant, discordia3a-2 discordia3b (dcd3a-2 dcd3b), and a semi-dominant mutant, Clumped tassel 1 (Clt1), which may have disrupted ATP hydrolysis. To determine how these mutations influence KATANIN's microtubule severing function, in vivo microtubule severing was assessed. Results show reduced microtubule severing frequency in dcd3a-2 dcd3b and homozygous Clt1 mutant plants, though not a total loss, suggesting other microtubule severing proteins may be functional in maize. dcd3a-2 dcd3b and Clt1 mutants have shorter stature and less elongated cells than their wild-type siblings. Abnormal cell division positioning was observed in dcd3a-2 dcd3b double mutant cells, primarily in asymmetrically dividing cells. Time-lapse imaging of dcd3a-2 dcd3b plants expressing a microtubule marker will pinpoint when asymmetric cell divisions go awry. Characterizing KATANIN function in maize will lead to a greater understanding of the impacts of microtubule dynamics and organization on plant growth and development.

Gene / Gene Models described: *ktn2, clt1*; GRMZM2G054715, GRMZM2G017305
Funding acknowledgement: National Science Foundation (NSF)

P96

Large-scale single-cell profiling of stem cells uncovers regulators of plant shoot development

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Stem cells are a rare population of cells in plant shoots that produce leaves, fruits and seeds, vital sources for food and bioethanol. Uncovering regulators expressed in these stem cells will inform crop engineering to boost productivity. Single-cell analysis is a powerful tool for identifying regulators expressed in specific groups of cells. However, accessing plant shoot stem cells is challenging. Recent single-cell analyses of plant shoots have not captured these cells, and failed to detect stem cell regulators like *CLAVATA3* and *WUSCHEL*. In this study, we finely dissected stem cell-enriched shoot tissues from both maize and arabidopsis for single-cell RNA-seq profiling. We used an optimized protocol to efficiently recover thousands of *CLAVATA3* and *WUSCHEL* expressed cells. A cross-species comparison identified conserved stem cell regulators between maize and arabidopsis. We also performed single-cell RNA-seq on stem cell overproliferation mutants in maize to find additional candidate regulators. Expression of candidate stem cell regulators was validated using spatial transcriptomics, and we functionally confirmed roles in shoot development. These candidates include a family of ribosome-associated RNA-binding proteins, and two families of sugar kinase genes related to hypoxia signaling and cytokinin hormone homeostasis. Our cross-species stem cell profiling provides a resource for mining stem cell regulators, advances understanding of shoot development, and opens avenues for manipulating diverse crops to enhance food and energy security.

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P97

Linking brace root development to function

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Nodal roots form the main root systems of a mature maize plant. Nodal roots consist of two types; crown roots that originate from below-ground nodes and brace roots from aboveground nodes. While previous research emphasizes the vital role of brace roots in providing stability and resistance to lodging, the specific impact of altering brace root development on other essential root functions remains unexplored. To investigate this, we first conducted RNA sequencing on stem nodes at various stages of brace root development to identify candidate genes. Our analysis identified SBP transcription factors among the differentially expressed genes. We show that SBP mutants such as unbranched 2 (ub2) unbranched 3 (ub3) and tasselsheath4 (tsh4) exhibit diverse brace root phenotypes, including variations in the number of roots per whorl, the number of whorls penetrating the soil, and brace root diameter. In addition, anchorage and nitrogen uptake capabilities were altered in these lines as well as in the dominant Corngrass1 (Cg1) mutant that overexpresses miR156 which targets SBP genes. Our results show that early in development when the contribution of brace roots to anchorage is minimal, root torsional stiffness is similar across different genotypes. Later, however, when brace root contributions are significant, mutants with smaller brace root diameters had reduced root torsional stiffness, while those with larger diameters were increased. Furthermore, Cg1 mutant brace roots took up more N15-labeled nitrogen than the wild-type, without showing preference for specific nitrogen types. Together, these data demonstrate that SBP genes impact brace root, anchorage, stiffness, and adsorption, functions that were not previously associated with these genes.

Gene / Gene Models described: *UB2, UB3 and TSH4*; Zm00001eb035030, Zm00001eb199880 and Zm00001eb316740
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P98

Maize *ETHYLENE INSENSITIVE3-LIKE* genes are central regulators of shoot growth

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The hormone ethylene plays a key role in plant development where it acts largely to inhibit growth. Ethylene is also a pivotal stress signal. In contrast to the wealth of knowledge regarding ethylene's roles in Arabidopsis, our understanding of its function in cereal crops is markedly limited. Here, we characterize mutations in maize *ETHYLENE INSENSITIVE3(EIN3)-LIKE1* (*ZmEIL1*) and *ZmEIL9* genes that encode co-orthologs of the Arabidopsis *EIN3/EIL1* 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. *Zmeil1;Zmeil9* double mutants are insensitive to the ethylene precursor ACC in growth assays. We utilized single-cell transcriptomics to identify tissue- and cell-specific genetic signatures in *Zmeil1;Zmeil9* and normal shoots with and without ACC. Transcripts of genes in hormone pathways and of genes involved in growth regulation differentially accumulate in various cell clusters between genotype and treatment conditions. We leveraged DNA Affinity Purification sequencing to identify genome-wide binding positions of ZmEILs. We found that many ZmEIL1 and ZmEIL9 binding sites overlap, suggesting co-regulation of a portion of putative target loci, including a proportion of genes that were identified as differentially expressed in our single-cell data. Our study shows that *ZmEIL* genes are central regulators of shoot growth and, by uncovering tissue-specific responses of the ethylene pathway, provides novel insights into our understanding of ethylene signaling in plants.

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P99

Maize developmental transcription factors affect and interact with *aberrant phyllotaxy1* in regulating phyllotaxy

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Regular patterned leaf initiation at the shoot apical meristem (SAM), or phyllotaxy, determines a plant's arrangement of leaves around a stem. In maize phyllotaxy, leaves are initiated successively on alternating sides of the plant, offset by 180˚. Among all plants, few mutants have been identified that influence phyllotaxy, suggesting the process is robust and functionally buffered. In maize, mutants for the *aberrant phyllotaxy1* (*abph1*) cytokinin signaling gene have altered phyllotaxy wherein leaves are instead initiated on opposite sides of the plant in pairs offset by 90˚. *abph1* mutants have an enlarged SAM which is proposed to result from complex interactions between the hormones cytokinin and auxin. We have identified transcription factor (TF) genes *ereb130*, *ereb184* and paralogs whose mutants result in altered phyllotaxy reminiscent of *abph1*. These TF mutants have relatively high penetrance in standard inbred lines in which *abph1* mutants are nearly normal. Expression analysis of *ereb130* mutants indicates a potential reduction in the biosynthesis of auxin, a hormone necessary for leaf initiation. Meristem size is unaffected in the inbred single mutants examined, yet the double mutants *ereb130; ereb184* or *ereb130;abph1* show similarly enlarged SAMs. Both double mutants show an increase in the total number of leaves initiated, yet this interaction is additive in *ereb* double mutants and synergistic in combination with *abph1*. These mutants allow for further elucidation and integration of hormones, gene expression, and morphology in the regulatory network controlling phyllotaxy. To further explore this, RNA-seq data from these various mutants is being combined with DAP-seq data for the EREB TFs to determine shared or independent changes in expression dynamics among these mutants that relate to the phyllotactic phenotypes. Given variation in SAM size and expression patterns among maize inbred lines, these mutants open a door to explore how diverse inbreds might differentially modulate leaf initiation.

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

P100 @kamilinyPlants

Making good connections: How transverse veins span the maize leaf vascular network

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The plant vascular system is a complex network which connects all organs. In maize leaves, the midvein, primary lateral, intermediate, and small veins are developed parallel to the direction of growth. Transverse veins, on the other hand, grow perpendicular to the other vein types, and therefore interconnect the entire leaf vascular system. We used a combination of plant anatomy tools and hormone reporters to observe the morphology and cellular development of transverse veins. A combination of diagnostic stains demonstrate that transverse veins may form between all parallel vein classes (midvein, lateral, intermediate, small), and like parallel veins, mature transverse veins consist of xylem, phloem and bundle sheath cell types. Although transverse veins may initiate connections between parallel veins which have already differentiated specialized cells, mature transverse veins display seamless connections with the bundle sheath, xylem vessel elements, and phloem sieve tubes of parallel veins, requiring cellular reorganization from these cells. Using the PIN1a-YFP marker line, early transverse vein formation is observed as single transverse anticlinal cell divisions followed by high PIN1a-YFP expression within paired spots along the procambium of parallel vascular bundles, flanking an incipient transverse vein. Cells in these spots are characterized by prominent nuclei and a denser cytoplasm than observed in surrounding procambial cells. Later, PIN1a-YFP is observed in a small file of cells that converges bidirectionally inward, derived from anticlinal divisions within leaf ground tissue cells. Our evidence suggests that PIN1a-YFP appears at the same time or after the first divisions which define the transverse vein, and DR5::erRFP is not detected in these cells. In contrast to parallel vein types, we conclude auxin transport and signaling are involved after an unknown vein recruitment signal, perceived prior to ground cell division. Ongoing work uses transverse veins as an experimental system for monocot vein ontogenesis via live imaging.

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P101

Nuclear membrane proteins and their functions beyond the nuclear membrane

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The nucleus is the characteristic organelle that makes eukaryotic cells distinct from archaea and prokaryotes. Nucleus contains genetic materials and protects itself by double layer membrane. Like any other membrane, double layer nuclear membrane includes proteins, and they are known as nuclear membrane proteins. In a dynamic cell, the nucleus is motile, and this movement is mediated by the interaction between nuclear membrane proteins and cytoskeleton. In a recent study, I have demonstrated that if this interaction between nuclear membrane proteins and cytoskeleton is interrupted at cellular level, nucleus fails to position correctly, and the future cell division follows the altered nuclear position. This study answers a long-standing biology question: The nucleus decides the future cell division site. In careful observation, this event highlights how nuclear envelope proteins regulate cell division, more precisely future division site. Interestingly, nuclear envelope breaks down during the cell division and re-appears in the daughter cell nucleus. What happens to the nuclear membrane proteins after the envelope breaks down? What is the function of nuclear envelope proteins during mitosis? The research in my lab tries to answer these fundamental questions using the *Zea mays* genetic and cell biology resources.

Gene / Gene Models described: *Mlks2*; Zm00001d052955 / Zm00001eb034900
Funding acknowledgement: Howard University start-up grant

P102

PEG-mediated transient gene expression in maize root protoplast

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Transient gene expression in plant protoplast has been used in gene functional characterization, protein subcellular localization, and protein-protein interaction. Plant protoplast isolation and transient expression methods have been reported in some plants, including Arabidopsis, tomato, apple, maize, etc. However, previous maize protoplast transformation methods were primarily done with mesophyll protoplast, or suspension-cultured cells. There is no report on protoplast transformation with maize root protoplasts. Here, we describe a PEG-mediated transformation for transient gene expression in maize root protoplasts to characterize transcription factors that may be involved in maize cuticle biosynthesis. There is limited reference data for maize root protoplast transformation, and we have tested several different transformation methods. We show that PEG-mediated transformation has the most consistent transformation efficiency and gene expression, but the transformation efficiency remains low. Our protocol provides a maize root protoplast transformation method for molecular and cellular biology research.

Funding acknowledgement: National Science Foundation (NSF)

P103

Parallel spindle genes restore haploid male fertility – removing a bottleneck in doubled haploid technology

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Plant breeding must be accelerated to meet the growing agricultural demands. Doubled haploid (DH) technology can accelerate plant breeding by reducing the time needed to produce inbred lines in two generations versus six or more generations with conventional breeding. The two main steps in DH technology are: haploid induction and subsequent DH production. In maize DH breeding, haploid inducers have been established and causal genes are identified. Haploid plants - intermediates between diploids and DHs - carry only one set of chromosomes leading to erroneous male meiosis I (MI) resulting in male sterility. Current protocols commonly use colchicine (a mitotic inhibitor) to mitigate the sterility to produce DH. This process is laborious, resource intensive and inefficient. Alternatively, haploid male fertility (HMF) has been observed in some maize gentoypes, barley, pummelo, rapeseed, rice, rye and wheat bread but no genes have been described. Haploid female fertility (HFF), however, is present to some extent in plants possibly involving mechanisms different from HMF. Previous research has shown that in diploid *Arabidopsis* mutant, *Atspo11-1* or *Atspo11-2*, chromosomes are unequally distributed during male MI, despite the presence of two sets of chromosomes. The result is also male sterility. Mutations in two individual genes, *Atps1-1* and *Atjas-2* can independently correct chromosomal distribution error of diploid *spo11-1*. These two mutants form parallel spindles (PS) in MII instead of the perpendicular spindles in wild type. We hypothesized that *Atps1-1* and *Atjas-2* mutations can correct erroneous MI in haploids. Herein, we demonstrate that mutations in the *ps* genes is sufficient to restore HMF in *Arabidopsis* with no impact on HFF. These genes are conserved across plant kingdom. Putative maize candidate genes have been identified and are currently being studied for their role in HMF restoration.

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P104

Phytoextraction potential of Quinoa and wheat grown under saline conditions as a measure for adaptation to salt stress

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Phytoremediation efficiency depends on the uptake mechanisms of soil contaminants by the plants. Phytoextraction is a potential alternative to diffuse the soil minerals in agriculture lands using high yielding crops, as these are expected to decontaminate the soil. Two crop plants *Chenopodium quinoa* and *Triticum aestivum* have been investigated for phytodesalination. Plants were exposed to stress at three salinity levels (5, 10, and 15 dsm $^{-1}$) for 28 days and the remediation efficiency of sodium, potassium, calcium, magnesium, and chlorine was evaluated. *Chenopodium quinoa*, with 23.96% efficiency, showed the highest potential for sodium uptake at the highest salinity levels. The highest magnesium and chloride uptake efficiencies were 24.52% and 18.41% in *Chenopodium quinoa* plants at the highest salinity level. The efficiency of phytoremediation of potassium in *Triticum aestivum* at the highest salinity level was 5.03 ± 2.04% and in *Chenopodium quinoa* was 10.33 ± 2.48%. Bioaccumulation of sodium, potassium, calcium, magnesium, and chloride was also investigated in the roots and shoots of plants. The efficiency of calcium uptake in *Triticum aestivum* decreased with increasing salinity levels and reached 14.97 ± 3.52%. Calcium uptake efficiency in both plants decreased with increasing salinity levels and reached 14.97 ± 3.52 and 20.85 ± 4.11%, respectively. The leaf area and dry weight of *Chenopodium quinoa* increased with increasing salinity levels. The highest transfer factor of 11.39 was observed in *Chenopodium quinoa* for calcium ions in the control sample. The results suggest that, in salinity areas, *Chenopodium quinoa* and *Triticum aestivum* should be grown alternately to improve the land reclamation, reduce salinity, and increase yields of wheat.

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P105

Plant regeneration using morphogenic regulators

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Plants possess an incredible degree of developmental plasticity and ability to regenerate. Plant regeneration can be generally achieved using tissue culture systems involving the manipulation of the hormones auxin and cytokinin. However, the ability to regenerate shoots is still a bottleneck in several plant species. Ectopic overexpression of plant morphogenic genes is a promising strategy for increasing the regeneration efficiency of recalcitrant plants, such as maize. Such morphogenic genes include the transcription factor BABYBOOM (BBM) and the transcription factor-cofactor complex GROWTH REGULATING FACTOR4-GRF-INTERACTING FACTOR1 (GRF-GIF), whose overexpression has been previously shown to improve regeneration in maize while avoiding pleiotropic effects. However, the mechanisms through which these factors promote shoot regeneration are not well understood. My project seeks to investigate the mechanisms of plant regeneration by using a previously developed transformation system overexpressing BBM and GRF-GIF (GGB system). To address this, I am performing a time course RNA-Sequencing experiment on wild type and GGB calli to assess differential expression of important stem cell development regulators. Moreover, I am examining auxin dynamics in GGB calli by visualizing the expression and localization of the PINFORMED1(PIN1) auxin efflux transporter using timelapse confocal microscopy. The characterization of gene expression and hormone dynamics in the GGB system will enable us to better understand and enhance the plant regeneration process. Improving plant regeneration will make plant transformation and agricultural biotechnology more efficient.

Gene / Gene Models described: *BABYBOOM, GROWTH REGULATING FACTOR4-GRF-INTERACTING FACTOR1*; Zm00001eb247080, Zm00001eb007630, Zm00001eb056050
Funding acknowledgement: National Institutes of Health (NIH)

P106

Regulation of maize ear development by a CLAVATA-related receptor complex

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Plant organ size and shape is controlled by meristem proliferation and differentiation. Meristem size is regulated by the CLAVATA (CLV)-WUSCHEL (WUS) signaling pathway, which involves an interplay between CLV receptors, their ligands, and the mobile transcription factor WUS. Maize mutants lacking CLV receptors have fasciated ears, with flattened tips and disordered kernel rows, a result of overproliferating meristematic cells. One such mutant, *fasciated ear3* (*fea3*), lacks a functional copy of a leucine rich receptor-like protein. Weak *fea3* alleles increase ear size without a compensatory loss in seed size, making this an attractive target for yield enhancement. The molecular mechanism by which FEA3 exerts its control on meristem size is unknown, as *FEA3* is expressed in a spatially distinct domain from other *CLV* receptors, and *fea3* interacts additively with other *CLV* receptor mutants.Intriguingly, a *CLV1* paralog, *ZmBARELY ANY MERISTEM 1D (ZmBAM1D*), is upregulated in *fea3* mutants. *FEA3* and *ZmBAM1D* expression overlaps in the center of spikelet meristems and the two proteins interact when co-expressed in *N. benthamiana*. While *bam1d* does not have an ear phenotype, *fea3;bam1d* mutants are more fasciated than *fea3* mutants, suggesting that the two genes interact. Furthermore, both FEA3 and ZmBAM1D perceive the same CLE peptide ligand. These observations suggest that FEA3 and ZmBAM1D may form a receptor-co-receptor pair. We are validating the interaction between ZmBAM1D and FEA3 and discovering additional *in vivo* interactors using proximity labeling with TurboID, which can better resolve transient protein-protein interactions compared to immunoprecipitation-based approaches. Since BAM receptors have pleiotropic roles in plant development and defense, comparing the proximity labeling interactome of ZmBAM1D and FEA3 may also help reveal how signaling specificity is achieved in different biological contexts. With a deeper understanding of how these receptors regulate meristem activity, we can more precisely engineer this process to enhance yield-related traits.

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P107

Regulation of meristem size by the *REL2*corepressor family

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The post-embryonic formation of the plant body proceeds in a sequential manner through the action of meristems, organized structures containing plant stem cells. Tightly coordinated meristematic regulation is required for proper development and reproductive success, eventually determining yield in crop species. In maize, the RAMOSA ENHANCER LOCUS2 (REL2) family of transcriptional corepressor proteins includes four members, REL2, RELK1 (REL2-LIKE1), RELK2, and RELK3. In an unbiased genetic screen for enhancers of the *rel2* mutant, we identified double mutants with fewer and shorter internodes, and enlarged female inflorescence meristems (IMs) carrying mutations in *RELK1*. We show that *RELK1* partially buffers the developmental defects of *rel2* mutants and actively compensates for the loss of *REL2* function. We therefore investigated the function and relationship among the various family members by also generating CRISPR-Cas9 induced mutations in *RELK2* and *RELK3*. Our analysis shows that *RELK* genes have largely redundant functions in the development and maintenance of various meristem types throughout the plant life cycle. Analysis of different triple mutant combinations revealed that *REL2*, *RELK1*, *RELK2*, and *RELK3* function redundantly during embryogenesis and in the formation and maintenance of the shoot apical meristem (SAM). Different double mutants demonstrated that ear formation requires *REL2* and *RELK3* while *REL2, RELK1* and *RELK2* are required for maintenance of ear IM. Overall, *REL2* functions as the essential gene, while the other members appear dispensable and can only partially compensate its loss. We are currently assessing to what extent activate compensation mechanisms are in play among the various family members. Expression analysis show that *REL2* and *RELK1* cooperatively regulate female IM development by regulating genes involved in redox balance, hormone catabolism, and differentiation ultimately tipping the meristem toward a molecular environment favorable to expanded expression of *ZmWUS1*, encoding a stem-cell promoting transcription factor. We also show that the loss of one functional copy of *REL2* can promote an increase in kernel row number across a diverse set of F1 hybrid combinations. Our findings reveal that the REL2 family of transcriptional corepressors maintains maize development from embryonic initiation to reproductive growth and can potentially be harnessed for increasing seed yield in a major crop species.We acknowledge funding from the National Science Foundation (IOS#2026561).

Gene / Gene Models described: *REL2, RELK1, RELK2, RELK3, WUS1*; Zm00001eb415530, Zm00001eb127680, Zm00001eb011010, Zm00001eb398420, Zm00001eb067310
Funding acknowledgement: National Science Foundation (NSF)

P108

Single-cell RNAseq analysis and dynamic modeling of leaf-angle variation in the maize leaf canopy

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Maize leaves comprise a proximal sheath surrounding the stem and a distal, photosynthetic blade. The blade/sheath boundary is demarcated by an epidermal fringe of ligule tissue and the multi-layered auricle, which forms triangular-hinges that create leaf-angle. Leaf-angle describes the bending of the distal blade away from the proximal sheath, a key architectural trait and a target for crop improvement. Multiple components influence leaf-angle, including auricle size, shape and wrapping, as well as the mechanical strength of the midrib. Candidate genes regulating leaf-angle have been identified by genetic, quantitative genetic, and transcriptomic analyses, enabling models of gene regulatory networks. Nonetheless, little is known about the control of leaf-angle variation in the lower-versus-upper canopy of maize plants. Here we describe a systems biology approach combining single-cell RNAseq (scRNAseq) analysis of the emerging ligule/auricle in lower-canopy and upper-canopy leaves, and dynamic modeling of gene regulatory networks, to probe the mechanism of leaf-angle variation in maize. Three inbred lines (EP1, B104 and B73) were sampled, each of which shows distinct variation in leaf-angle within the canopy. Specifically, upper-leaves have increased leaf-angle (i.e. are flatter) in inbred EP1, but are gradually more upright in inbred B73. In contrast, inbred B104 displays equivalent leaf-angle in the upper and lower canopies. We used single-cell RNAseq to profile the transcriptomic landscapes of early-staged ligule/auricles from the lower (below ear leaf) and upper canopy (above ear leaf) in the three inbred lines. A total of 25 cell-clusters were identified in the Uniform Manifold Approximation and Projection (UMAP) of all scRNAseq samples. Inbred lines showed cell-cluster separation. Comparisons of upper and lower canopy revealed cell-cluster separation within all inbred lines, although the degree of separation is less in B104. Finally, we introduce a dynamic model to systematically describe the mechanisms of maize ligule/auricle development, and its variation in the maize leaf canopy.

Funding acknowledgement: National Science Foundation (NSF)

P109

The maize preligule band is subdivided into distinct domains with contrasting cellular properties prior to ligule outgrowth

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During development, boundaries separate cell populations with different fates. In plants, boundaries have been shown to be regions of restricted growth that make critical contributions to morphogenesis. The maize leaf provides an opportunity to study a relatively accessible boundary region called the preligule band (PLB). The PLB, which forms at the blade-sheath boundary, gives rise to two distinct structures, the ligule, and the auricles. The ligule, a membranous structure that covers the gap between consecutive leaves, arises exclusively from the PLB epidermis. The auricles are wedge-shaped structures that develop immediately distal to the ligule and contribute to blade angle. Here, we characterize ligule development in terms of epidermal topography, cell geometry, division orientation, and cell wall rigidity. The ligule development program is linked to cell wall rigidification within the PLB. Differential growth of cells in the sheath, PLB, and blade contributed to the formation of a shallow ridge within the PLB, which ultimately produces the ligule fringe. Subsequent changes in division plane orientation, cell geometry and cell wall stiffness are consistent with the partitioning of the PLB into at least two distinct zones prior to ligule outgrowth. Our findings lay an important foundation for future experiments and highlight the maize ligular region as a promising system for studying the relationships between gene expression, biomechanics, and growth during plant organogenesis.

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P110

The Wisconsin Crop Innovation Center: a public resource for maize transformation and gene editing research

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The Wisconsin Crop Innovation Center (WCIC), part of the University of Wisconsin – Madison, is a ~100,000 ft2 facility operating as the largest public sector fee-for-service plant transformation laboratory in North America. Following a conventional protocol, the WCIC routinely genetically transforms the elite maize inbred LH244, a B73-related line recently released from intellectual property protection. In the near future, the WCIC will offer as a service a developmental gene facilitated transformation path which will increase the potential to transform more maize inbreds. Over-expression and/or CRISPR/Cas9-based gene editing is currently available in LH244. All transformation starts with a contract negotiation between UW and the client's institution and upon execution, the transformation pipeline can begin. Typically, LH244 transformation starts with binary plasmid vector design and assembly as a service within the WCIC. In the near future, DIRECTCLONE plasmids, which enable simplified integration of user genes/guide RNA will be available. Upon the creation or delivery of a transformation vector, Agrobacterium stocks are created and then used to make transgenic maize. Regenerated plantlets from culture are sent to the greenhouse for plant analysis of the selection marker. For overexpression vectors, copy number services are available to identify single locus events. Plants will be advanced and selfed or outcrossed to generate T1 seed. WCIC will even advance the T1 seed generation and screen it to grow homozygous T1 plants to bulk T2 seed for direct trials, and/or the creation of hemizygous transgenic hybrid T2 seed from the T1 plants outcrossed to a second inbred parent. For editing vectors, T0 leaf tissue samples are available to be shipped for the client to evaluate editing efficiency and a seed-expressed color gene marker is included to assist segregation of the edit from the editing locus in the T1 seed. In the near future, WCIC will offer edit evaluation in-house. All transgenic seed is available to be shipped to the client after completion of USDA-APHIS permits required for interstate seed shipment.

P111

The boron deficiency response during primary root development of maize

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The micronutrient boron is essential for plant development. Boron, in form of boric acid, is taken up by plants from the soil. Therefore, soil boron deficiency, which is a global problem, negatively affects crop yield and quality. While studies on boron deficiency-induced defects in maize have focused on shoot parameters, knowledge regarding the impact of boron deficiency on maize roots and the underlying causes is scarce. Most cellular boron is located in cell walls, crosslinking the pectic subunits rhamnogalacturonan II. Recent findings highlight boron deficiency-induced cell division defects and boron-phytohormone relations, opening up intriguing avenues to reveal potential cell wall-independent functions of boron. The experimental induction of boron deficiency is challenging, as boron is ubiquitous. Phenylboronic acid (PBA), which is structurally similar to boric acid, is proposed to compete with boric acid for boron binding sites, therefore inducing boron deficiency. Our study aims to characterize boron deficiency-induced defects in the maize primary root using PBA.
We characterized the boron deficiency-induced defects in maize roots through time course experiments and compared these defects to those induced by PBA. PBA treatment and boron deficiency (using boron-free Hoagland medium) exhibited similar defects with different severity. Both treatments significantly reduced the lateral roots density and primary root length compared to conditions with adequate boron. In addition, histology results revealed that PBA treatment significantly increased the thickness of the primary root. The boron deficiency-induced reduction in lateral root density was correlated with a reduction in lateral root primordia numbers, indicating defects in lateral root initiation. The potential connection to the auxin cascade and cell division for these defects was assessed through time course experiments with auxin biosynthesis mutants and transgenic marker lines. Our results pinpoint boron-deficiency induced cellular and molecular defects, shedding light on the functions of boron during primary root development of maize.

Funding acknowledgement: German Research Foundation (DFG)

P112

The cis-regulatory evolution of GRASSY TILLERS1 (GT1) over deep time

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The evolution of cis-regulatory regions is critical for evolutionary divergence in gene function. One class of developmental genes that may have shifted in function following cis-regulatory change are the class I HD-ZIP transcription factor genes. Class I HD-ZIPs control multiple developmental programs in flowering plants and have deep conserved roles in grasses like maize, barley, and wheat. For example, GRASSY TILLERS1 (GT1) is a pleiotropic domestication gene involved in repressing growth in multiple developmental contexts, including in lateral buds, roots, leaves, and floral organs. A cis-regulatory region upstream of GT1 (prol1.1) underlies a major QTL for ear number (prolificacy). To dissect the function and evolution of prol1.1, we have traced the evolution of conserved non-coding sequences in the grasses, and are editing the GT1 promoter in Zea mays and Brachypodium distachyon to determine how GT1's pleiotropic functions are regulated and conserved in the grass family. RNA in situ hybridization in Zea mays and Setaria viridis suggest that GT1 homologs have conserved expression patterns in degenerating tissues. Our work will help us understand the evolution of this important, pleiotropic domestication gene, and precisely modulate gene expression in the grasses.

Gene / Gene Models described: *GT1*; Zm00001eb007950
Funding acknowledgement: National Science Foundation (NSF)

P113

The flowering phenotypes of temperate and tropical maize grown in short and long day field environments

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The critical transition in the maize life cycle involves a shift 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 pivotal stage occurs early in development and is influenced by a combination of internal cues and external signals, such as photoperiod and temperature. The timing of the floral transition significantly impacts flowering, the stage at which the plant releases pollen and develops silks—a crucial adaptive trait subject to selection. Maize varieties adapted to temperate latitudes exhibit photoperiod insensitivity, flowering consistently under both short and long day conditions. In contrast, tropical maize, retaining much of the photoperiod sensitivity of its progenitor teosinte, requires short days 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 two consecutive years—Hawaii (short days) and Iowa (long days). This poster illustrates how the growing environment influences the timing of SAM reprogramming, total plant leaf count, and the days to flowering in these genotypes. Our analysis also explores the interplay between photoperiod and temperature on flowering timing. We sampled for gene expression analysis and currently, we are in the process of analyzing the diurnal expression patterns before and after the flowering transition under field conditions under short days. Unraveling how these genotypes respond in diverse environments lays the foundation for future investigations into the molecular mechanisms distinguishing photoperiod-sensitive from photoperiod-insensitive maize varieties.

Funding acknowledgement: National Science Foundation (NSF)

P114

The genetic basis of callus development and resources for genetic transformation of maize

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Callus induction is a key step for genetic transformation and regeneration of maize. Two types of calli, type I and type II, are derived from maize tissue culture. Type II grows faster and is more responsive for regeneration. The degree of type II callus induction is dependent on genotype, although the mechanisms and genetic basis are poorly understood. A188 is a regeneration-amenable inbred line used for genetic transformation, while B73 is a recalcitrant inbred line. A188 and B73 have high and low type II callus induction rates, respectively. Analysis of type I and II calli from a B73xA188 F2 population through single callus genotyping and pooled callus RNA-seq identified quantitative trait loci (QTLs) for callus type at chromosomes 2, 5, 6, 8, and 9. The analysis also identified differentially expressed genes (DEGs) between the two callus types. Currently, we are examining the impact of the DEGs on callus development by ectopic gene expression in maize immature embryos. At the same time, the quantification of the callus type of double haploids (DHs) from the cross between A188 and B73 led to the selection of highly culturable DH lines, providing a new genetic resource for maize transformation.

Funding acknowledgement: National Science Foundation (NSF)

P115

The heterofertilization and kernel abortion phenotypes in maize gex2 mutants are consistent with differential fertilization recovery of egg and central cells.

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Sperm cells of flowering plants accomplish double fertilization via attachment and subsequent fusion of sperm cells to the egg cell and polar nuclei in the embryo sac, giving rise to the embryo and endosperm respectively. Mutations in both the Arabidopsis and maize GEX2 orthologs are associated with reduced mutant transmission, and an increased production of aborted kernels when transmitted through the male; likely due to defects in gamete attachment. To better understand GEX2 function, we have characterized phenotypes associated with two different mutations in the maize gex2 gene caused by insertion of an engineered GFP-marked transposable element. Although both insertions interrupt GEX2 coding sequence, the alleles are associated with a statistically significant difference in transmission defect severity. We are using RT-PCR to investigate whether the less severe phenotype is linked to alterations in splicing or protein structure.Use of anthocyanin markers indicates that the most severe gex2 allele is associated with an increased rate of heterofertilization, where the egg cell and central cell are fertilized by different pollen tubes. Genotyping the embryo and endosperm of heterofertilized kernels shows 88.2% are associated with gex2 fertilization of the egg cell. Conversely, aborted kernels from male gex2 crosses typically develop endosperm without embryo, suggesting that these abortions are a result of single fertilization of the central cell. Taken together, these data suggest that gex2 sperm are associated with high rates of single fertilization, with fertilization recovery rescuing those associated with the egg but not central cell. To further investigate the gex2 heterofertilization phenotype, we adapted the pollen preservation approach of Shoemaker et al. 2022 to a double pollination protocol that facilitates measurement of heterofertilization rate. Comparison with previous results will allow validation of this new method, as well as determination of whether the different gex2 alleles also differentially influence heterofertilization.

Gene / Gene Models described: *gex2*; GRMZM2G036832, Zm00001eb099800
Funding acknowledgement: National Science Foundation (NSF)

P116

The maize *fusedleaf1* mutant

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In order to feed the Earth's ever-increasing population without compromising its remaining wild areas, agricultural productivity desperately needs to increase. This is especially poignant in cereal crops, which provide the largest source of global calories and yet remain broadly understudied compared to their eudicot relatives. Maximising the yield of these staple crops will require a greater understanding of the networks underpinning their development, including, crucially, the formation and development of their organs; one way to find genes important for such development is through mutant analysis. A fundamental step in organ development is the delineation of the differentiating organ cells (primordium) from the pool of stem cells (meristem) via the formation of a boundary region. Here, we present a new mutant in maize in which this boundary is defective both between primordia and between primordia and the meristem, called *fused leaf 1*.

Funding acknowledgement: European Research Council (ERC)

P117

The role of the plant growth hormones auxin and cytokinin in maize mutants lsn1 and Hsf1

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In this experiment, we sought to understand hormonal regulation in maize mutants large scutellar node1 (lsn1) and Hairy sheath frayed1 (Hsf1). The mutant lsn1 is characterized by abnormal vein patterning, a flattened and enlarged root tip, as well as a short shoot, and a short root with few lateral roots. The lsn1 phenotype is reported to be caused by defects in auxin transport which we hypothesize results in accumulation of auxin in the root tips stunting growth. We theorize that this would lead to lower levels of cytokinin in the root tips. The mutant Hsf1 is also distinguished by an abnormal phenotype, with hairs present on the surface of the leaves. The roots have been reported to be shorter than normal with fewer lateral roots. Hsf1 has a higher level of cytokinin signaling, and therefore we hypothesize that there would be lower levels of auxin present in the root tips. To test our hypotheses, we crossed the two mutants together to examine the double mutant phenotype. As auxin and cytokinin often act in opposition to each other, we predicted that the the double mutant would have a more normal phenotype. Come to the poster to find out what happened instead. This research expands upon existing research on these plant growth hormones, and how they affect root development.

Funding acknowledgement: National Science Foundation (NSF)

P118

Tracking S-phase progression: Sequential dual labeling of replicated DNA with EdU and BrdU in maize root tips

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Monitoring the progression of DNA replication can give insights into S phase and overall cell cycle kinetics. For this purpose, the thymidine analogs, 5-ethynyl-2′-deoxyuridine (EdU) and 5-bromo-2′-deoxyuridine (BrdU), have been used extensively to label newly replicated DNA during S phase. Combining both analogs in a sequential EdU followed by BrdU dual-pulse opens new opportunities for further defining S-phase kinetics using less experimental material. We adapted procedures for EdU-BrdU dual pulse labeling from mammalian cell lines and incorporated them with our published procedures for single-labeling with EdU in maize root tips for analysis by flow cytometry and fluorescence microscopy. Key technical optimization steps will be presented, including appropriate controls and how to monitor and balance the optimal DNA denaturation for BrdU antibody detection while maintaining sufficient double-stranded DNA for DAPI intercalation and DNA content measurements. This approach can be applied with various labeling times to enable new questions about different spatio-temporal aspects of S-phase progression. For example, by first labeling with EdU to identify cells in S phase, followed by a 1-hour thymidine chase, and then labeling with BrdU, we can determine how many cells have exited S phase at a later time point. By quantifying these populations with a flow cytometer, S-phase duration can be estimated from a single sample. In contrast, the EdU-only approach required lengthy time courses and much more plant material to estimate S-phase duration. In addition, combining dual labeling with fluorescence activated nuclei sorting (FANS) allows for the characterization of cytologically distinct labeling patterns separated by defined time intervals within single nuclei, revealing aspects of DNA replication progression.

Funding acknowledgement: National Science Foundation (NSF)

P119

Tradeoffs, balance and drift: the genetics of paralogous compensation in the maize meristem

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Evolutionary innovations are often achieved by co-opting existing molecular structures to perform new functions, a concept commonly referred to as "molecular tinkering". Gene duplication is a powerful source of biological innovation, giving rise to duplicates (hereafter, paralogs) that undergo diverse fates and drive evolutionary change. Redundancy between paralogous genes is an intriguing outcome of duplicate gene evolution, and its maintenance over evolutionary time has long been considered a paradox. Genetic studies in yeast and plants have suggested that the ability of ancient redundant duplicates to compensate for dosage perturbations resulting from a loss of function depends on the reprogramming of gene expression, a phenomenon known as active compensation. Our research work focuses on the maize trehalose-6-phosphate phosphatases RAMOSA3 and TREHALOSE PHOSPHATE PHOSPHATASE 4, two important meristem development regulators, as a model for studying the reprogramming of paralogs. By using quantitative imaging, chromatin accessibility assays, and promoter editing our work is investigating the hypothesis that cis-regulatory elements control paralogous compensation by binding to factors that regulate transcription. Understanding the transcriptional mechanisms of reprogramming among duplicated genes could allow us to fine-tune traits controlled by redundant paralogs, and improve the predictability of gene editing outcomes.

Gene / Gene Models described: *TPP4, RA3*; Zm00001eb194210, Zm00001eb327910
Funding acknowledgement: National Science Foundation (NSF)

P120

Transcriptomic profiles of developing meristems across sorghum accessions reveal nuanced regulatory pathways towards panicle morphology

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Gene regulatory networks (GRNs) are composed of coding and non-coding DNA elements that control gene expression. They have become an essential aspect towards crop improvement by revealing modules that influence stress response, development, and plant evolution. Such GRNs are best created through multi-omics approaches in specific tissues to strengthen candidate genes and loci for functional characterization and ultimate incorporation into breeding programs. Combining transcriptomics, transcription factor (TF) binding profiles, and other genomic metrics, we construct dense sorghum GRNs to understand conserved and divergent modules that influence inflorescence development across multiple Sorghum Association Panel accessions, including breeding, non-breeding, and conversion lines. Developmentally crucial gene sets can still show notable differences in expression across inchoate tissue stages, suggesting a level of genetic redundancy or plasticity exists towards creating similar panicle morphologies. Additionally, TFs that are known regulators of inflorescence meristem progression in monocots, like Bearded Ear1 and Tassel Sheath 4, have different binding profiles in the promoter regions of these meristematic gene clusters and also indicate which TFs could have a more promiscuous regulatory purview compared to others. Finally, comparing the sorghum results with maize transcriptomic profiles also revealed which orthologous genes are conserved in their regulation through different TFs between maize and sorghum.
This project was funded by the USDA-ARS award number 8062-21000-044-000D.

Gene / Gene Models described: *TSH4, BE1*; SORBI_3002G247800, SORBI_3004G281000
Funding acknowledgement: United States Department of Agriculture (USDA)

P121 @caldwed

Uncovering the infection strategy of Phyllachora maydis during maize colonization: A comprehensive analysis

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Tar spot, a disease caused by the ascomycete fungal pathogen Phyllachora maydis, is considered one of the most significant yield-limiting diseases of maize (Zea mays L.) within the United States. P. maydis may also be found in association with other fungi, forming a disease complex which is thought to result in the characteristic fish eye lesions. Understanding how P. maydis colonizes maize leaf cells is essential for developing effective disease control strategies. Here, we used histological approaches to elucidate how P. maydis infects and multiplies within susceptible maize leaves. We collected tar spot-infected maize leaf samples from four different fields in northern Indiana at three different time points during the growing season. Samples were chemically fixed and paraffin-embedded for high-resolution light and scanning electron microscopy. We observed a consistent pattern of disease progression in independent leaf samples collected across different geographical regions. Each stroma contained a central pycnidium that produced asexual spores. Perithecia with sexual spores developed in the stomatal chambers adjacent to the pycnidium, and a cap of spores formed over the stroma. P. maydis reproductive structures formed around but not within the vasculature. We observed P. maydis associated with two additional fungi, one of which is likely a member of the Paraphaeosphaeria genus; the other is an unknown fungi. Our data provide fundamental insights into how this pathogen colonizes and spreads within maize leaves. This knowledge can inform new approaches to managing tar spot, which could help mitigate the significant economic losses caused by this disease.

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P122 @_DianaRuggiero

Understanding leaf vascular density through quantitative genetics and high-throughput phenotyping

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Efficient C_4 photosynthesis requires high vein density to shuttle carbon from mesophyll to vascular bundle sheath cells. Maize leaves have several vein subtypes (lateral, intermediate, small, and transverse) defined by their sequence of development and spatial configuration. Amongst these, small veins make up most of the vascular tissue in the leaf and preferentially develop in the photosynthetically active blade. We are using natural diversity within the Wisconsin Diversity Panel (WiDiv) to identify genes influencing vascular density. We collected 6000+ leaf samples from 750+ WiDiv lines over three field seasons. To quantify microscopic vascular traits on a large scale we built a deep-learning phenotyping system that estimates subtype-specific vein density in images of cleared leaf tissues. The system implements a U-Net convolutional neural network (CNN) architecture for semantic, pixel-by-pixel image segmentation and vein-subtype classification. Because leaf physiology and vein density vary over leaf compartments, we trained a second U-Net model to segment leaf images into sheath, auricle, and blade, allowing for compartment specific phenotyping and GWAS. We used activation mapping and 'DeepDream'-style feature visualization to show how the model interprets the leaf images at different levels of its architecture, showing that early model layers conduct fundamental image processing such as edge detection while later layers consider the spatial configuration of multiple vein subtypes. We used our data to perform correlation analysis between vein phenotypes and traits from other datasets. Our system also detects widespread 'bundle-sheath fusions', where ectopic bundle sheath cells appear between small veins in place of mesophyll cells, violating the spacing required for C_4 photosynthesis. We used FarmCPU to conduct a preliminary vein trait GWAS on 14M+ publicly available SNPs, which revealed a polygenic genetic architecture for leaf vein phenotypes. These candidate loci will guide future studies in C_4 vascular density and arrangement.

Funding acknowledgement: National Science Foundation (NSF)

P123

Unravelling the role of LIGULELESS2 in maize

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Life on earth is marked by an incredible diversity in form and function, but how this diversity is defined and coordinated throughout development remains a fundamental question in biology. Organ shape poses an attractive target for crop improvement given that it can profoundly influence plant productivity, but how organ shape is controlled remains unclear. Boundary formation between and within organs is crucial for shape determination. These boundaries are defined during the earliest stages of development, when meristematic cells begin differentiating into distinct domains. Determination and maintenance of these domains influences growth patterns within the primordia to direct how an organ will be shaped. However, the gene regulatory networks underlying domain patterning and boundary specification and how they are modified to generate morphological diversity remain unknown. In maize the BZIP transcription factor LIGULELESS2 (LG2) is thought to have a key role in defining boundaries between domains in different organs. *lg2* mutants lack a clear boundary between the leaf sheath and blade, the tassel has fewer branches, and there are defects in bract formation. How LG2 functions during the development of these different organs, and specifies boundaries is unclear. To address this, we are combining RNAseq, ChIPseq and CoIP to build the gene regulatory network that LG2 acts in. Through comparing the GRN between organs we hope to identify both core components, providing insight into how boundaries may be specified and maintained in growing organs, and key organ-specific changes that may be responsible for morphological differences.

Funding acknowledgement: ERC

P124

Vasculature connection in maize "Twin-Grafts"

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Grafting is a technique that has been used in agriculture for millennia to combine two genetically distinct crop varieties to create a plant with maximally 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 by preforming the grafting in pre-germinated seeds and kernels. Indeed, we have developed a protocol and shown the ability of *Zea mays* to form a 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 phloem-xylem connectivity established 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 shoot via the xylem. Gamma ray counting and autoradiography were used for these measurements. In subsequent studies, shoot/shoot xylem connectivity will be examined using radioactive 2-deoxy-2-[^{18}F]fluoro-D-glucose administered hydroponically through a select set of nodal roots connected to just one of the shoots. 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 in the future. 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

P125

WHETSTONE1 (*WTS1*), a single dominant locus from the W22 background suppresses *Wavy auricle in blade2* (*Wab2*)

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The maize leaf has three morphologically distinct domains: a flat photosynthesizing blade, a rigid sheath grasping the stem, and a ligule with flexible auricle connecting blade to sheath. We previously showed *Wavy auricle in blade2* (*Wab2*) is a semi-dominant leaf mutant which produces ectopic auricle-like tissue in the blade. Expression of *Wab2* is highly variable in heterozygotes. We attempted to identify the underlying genetic mechanism by crossing *Wab2* introgressed into the B73 background with W22. B73-*Wab2* x W22 F1 hybrids did not express the *Wab2* phenotype (N=40), demonstrating that one or more dominant suppressor loci modify expression of *Wab2* in W22. We characterized the F2 generation to understand the genetic architecture of this suppression and identify causative loci by bulk segregant analysis (BSA) mapping-by-sequencing. Although genotyping via a cleaved amplified polymorphic sequence (CAPS) marker revealed that inheritance of the *Wab2* allele matched Mendelian ratios (N=142), phenotypic ratios displayed significant suppression of the *Wab2* allele in both heterozygotes and homozygotes. Here we used chi-squared goodness-of-fit tests to show that a single dominant suppressor, is the most parsimonious explanation for non-Mendelian variation in auricle-like tissue phenotypic expression. Because of its function in restoring wildtype blade morphology, we refer to this locus as *WHETSTONE1* (*WTS1*). When the W22 allele, *WTS1-W22* is present *Wab2* is suppressed, whereas the B73 allele, *WTS1-B73* allows *Wab2* to be expressed. Using our genotypic and phenotypic data, we collected DNA from pools of 51 *Wab2* suppressed individuals and 51 *Wab2* expressed individuals from this F2 population to identify *WTS1* through BSA mapping-by-sequencing with an allelic distance approach. Ongoing work will explore molecular genetic interactions between *Wab2* and *WTS1* in driving maize leaf morphology. Potential future steps include fine mapping and functional validation using gene knockout or overexpression to confirm the role of *WTS1* in leaf morphology.

Funding acknowledgement: National Science Foundation (NSF)

P126

Wavy Auricle in Blade2 (Wab2) overexpresses tcptf15 and is suppressed by one copy of wavy auricle in blade1 (wab1) loss-of-function

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The maize leaf is separated into three genetic domains: a blade optimized for photosynthesis, a sheath wrapped tightly around the stem that provides structural support, and a ligule/auricle that acts as a hinge bridging the blade and sheath. We have been characterizing Wavy Auricle in Blade2 (Wab2), a maize leaf mutant that produces ectopic clusters of auricle-like tissue in the blade. Wab2 exhibits incomplete dominance and variable expressivity as a heterozygote. Histology of the Wab2 shoot apex showed multiple bands of actively dividing cells as early as the fifth leaf primordium (P5) from the meristem, resembling blade-sheath boundary tissues where the wild-type auricle forms. Bulk segregant sequencing identified a rough mapping interval on chromosome 4. RNA-seq analysis showed a predicted TCP transcription factor, tcptf15 (Zm00001eb179560), is 100-fold overexpressed in Wab2 mutants and the only differentially-expressed gene within the mapping interval. As the dominant allele of Wavy Auricle in Blade1 (Wab1-R) was previously shown to be caused by an overexpressed TCP transcription factor, tcptf15 has become our primary candidate gene for Wab2. To demonstrate tcptf15 is necessary for the Wab2 phenotype, we designed 4 sgRNAs against the locus that assemble using Gate Cloning, to create a revertant loss-of-function allele in the Wab2 background by CRISPR/Cas9. Double mutants between Wab2 and multiple wab1 loss-of-function alleles show wab1 is epistatic to Wab2. Unexpectedly, plants heterozygous for wab1-rev and wab1-MA also suppressed the Wab2 phenotype. We hypothesize that physical interaction between Wab2 and DNA-binding defective wab1-rev or wab1-MA proteins may explain heterozygous suppression. Future work will examine potential interactions between these proteins and place Wab2 within a genetic pathway for leaf development. There are still many questions left to be answered as we press forward in our study of this mutant.

Gene / Gene Models described: *Wab2, tcptf15, Wab1*; Zm00001eb179560

P127

*Zm*CAND1 is hyposensitive to auxin treatment and controls maize growth and development

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The phytohormone auxin controls plant growth and development. Auxin treatment of developing seedlings results in a strong inhibition of root growth. A concentration curve was performed with the synthetic auxin, 2,4-dichlorophenoxyacetic acid (2,4-D), on B73 maize seedlings grown in the dark. A concentration of 25 µM resulted in 85% reduction in root length. To identify genes involved in auxin perception and sensitivity we screened a set of B73-EMS M2 families treated with 25 µM 2,4-D and discovered one family that segregated seedlings with long roots and a reduction in nodal roots compared to wild-type controls. These insensitive mutants segregated in a recessive fashion and had altered growth and development under light grown conditions. The 2,4-D insensitive mutants had fewer leaves and were 54% shorter than wild-type controls. Tassel architecture was also affected as the mutant tassels were 51% shorter in length and had reduced tassel branching. To map the causative mutation, an F2 mapping population was created with Mo17. Bulk-segregant analysis and next generation sequencing was performed on a pool of 2,4-D insensitive mutants. A map region on chromosome 4 was identified by mapping SNPs between the mutant sequence pool and B73 reference genome. Only a single high effect transition mutation was identified in the *cullin-associated and neddylation-dissociated1* (*cand1*) gene resulting in a premature stop codon. The CAND1 protein is responsible for recycling the AUXIN-RELATED F-BOX (AFB) proteins involved in auxin perception. Continued work is underway to further characterize the mutant phenotype and identify a second allele.

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P128 @CraigCowling01

ZmPILS6 is an auxin efflux carrier required for maize morphogenesis

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The phytohormone auxin is essential for regulating plant growth and development. Proper spatiotemporal auxin distribution is required to maintain stem cell populations and promote cellular differentiation during organogenesis. Auxin transporters from the PINFORMED1 (PIN1) and ABC families are required for maize development, but the roles of the evolutionarily ancient PIN-LIKES (PILS) proteins are not well understood. Free indole-3-acetic acid (IAA, or "auxin") levels within the primary root are asymmetrically distributed, suggesting that this pattern is established by regulated transport and/or biosynthesis. Using reverse genetics, we have identified two ER-localized PILS proteins that influence auxin transport in roots. Knockdown alleles of *PILS6* in maize display altered auxin response in seedlings and impaired vegetative development, including reduced shoot height and crown root architecture. *pils6* roots have extensive proteome and phosphoproteome remodeling compared to W22, including extensive changes to hormone signaling and cell proliferation related proteins. PILS6 protein interactors were predicted using this proteomics data and Weighted Gene Co-expression Network Analysis (WGCNA). Within the PILS6 co-expression network are kinases, trafficking proteins, and other membrane transporters. Based on these findings, a working model for the roles of PILS6 in driving maize root and shoot formation will be presented, which may inform strategies for generating desirable architectural traits.

Gene / Gene Models described: *pils6*; Zm00001d043083
Funding acknowledgement: National Science Foundation (NSF)

P129

tasseless5 (*tls5*) affects plant height and reproductive development in maize by regulating internode elongation and tassel emergence.

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All flowering plants, including maize, form their plant body in repetitive units called phytomers, which consists of a node, an internode, leaf and an axillary bud. Internodes have meristematic tissue called intercalary meristems at their base which enable the stem to elongate after the floral transition. Intercalary meristems divide and the cells elongate which leads to internode elongation and increase in plant height. Any defects in intercalary meristems will disrupt internode elongation and affect plant height. Through an EMS mutation screen in maize, we identified the *tasseless5* (*tls5*) mutation which has defects in internode elongation in various parts of the stem, leading to reduced plant height. Additional defects include short tassels with fewer spikelets and branches and the tassels fail to emerge out of the whorl most of the time, hence the name *tassel-less*. Furthermore, multiple ears are visible which are small, compared to the normal maize ears. We have mapped the candidate region to chromosome 9 and are using a whole genome resequencing approach to identify possible candidate genes. Identifying the *tls5* gene and characterizing its function will provide insight into intercalary meristem development in maize internodes and uncover the additional role of the gene in maize reproductive development.

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P130 @MaizeGDB

MaizeGDB: AI-driven resources for maize

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In genetics and genomics, artificial intelligence (AI) and machine learning (ML) are increasingly being used in new and transformative roles. These technologies can now predict and design protein structures, generate interaction networks, assign functional annotations, and decipher the outcomes of mutations. These outcomes provide new opportunities for model organism databases to use AI/ML for high-throughput analysis and knowledge discovery. However, it also provides challenges in integrating predicted and generative results with existing high-quality experimentally determined datasets. MaizeGDB has embraced these innovations, offering a suite of AI/ML-driven tools tailored for maize research. Some of the new resources include (1) the Maize Feature Store, a centralized repository formatted for gene-based feature utilization in machine learning applications; (2) PanEffect, a tool to visualize potential effects of missense variants, considering both the B73 reference genome and the maize pan-genome; (3) Protein Structure Resources with tools like visualize, search, compare protein structures. Furthermore, MaizeGDB has developed several methodologies for functional annotations. MaizeGDB now has an AI data center to organize and provide a central resource to find these tools and datasets.

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P131 @MaizeGDB

A unified VCF dataset from 1,500 diverse maize accessions and resources to explore the genomic landscape of maize

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Maize has a wealth of phenotypic and molecular diversity, exceeding that of most model organisms and a great many wild and cultivated plants. Nucleotide diversity, including SNPs and small indels, as well as more complex structural variants such as translocations and inversions, are frequently used to estimate polymorphism in a population. Efforts to represent nucleotide diversity in maize have ranged widely in scope (e.g., the first HapMap project in 2009 captured 3.3 million SNPs and indels in 27 diverse maize inbred lines, and a 2023 project identified approximately 366 million segregating and 46 million high-confidence variants in 1,515 resequenced maize lines across maize wild relatives, landraces, and tropical and temperate lines). However, because of the divergence in pipelines and reference genome versions used in these studies, comparisons across data sets are difficult. Differences in reference genome versions, read mapping algorithms and parameters, and variant calling can make it difficult to know why variants exist in one data set but not another, and can lead to differing positions for the same variant. In order to address these continuity issues, The Maize Genetics and Genomics Database (MaizeGDB – https://www.maizegdb.org) has collaborated with researchers in the maize community to offer variant data through a standardized haplotype-calling pipeline that uses BWA-MEM and Sentieon's Haplotyper and GVCFtyper against version 5 of the B73 reference genome. The first release of the dataset was generated using 1,498 resequenced lines from 10 projects and includes a diverse set of inbred lines, landraces, and teosintes. The data set was filtered for mapping quality, coverage, and linkage disequilibrium, and annotated based on variant effects relative to the B73 RefGen_v5 gene annotations. MaizeGDB created a web tool to filter, visualize, and download genotype sets based on genomic locations and accessions of interest. MaizeGDB plans to host a regularly updated version of these resources as additional resequencing data become available, with plans to expand to all publicly available sequence data.

Funding acknowledgement: United States Department of Agriculture (USDA)

P132

MaizeMine: Tools for exploring genomic datasets from the maize research community

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Over twenty years of maize genomics research has created a large and diverse collection of high-quality data sets characterizing the maize genome. This has given rise to the need for specialized tools that allow researchers to integrate their data sets with publicly available genome annotation data as well as published community datasets. MaizeMine (http://maizemine.maizegdb.org), a component of MaizeGDB (http://maizegdb.org), aims to meet this need by integrating data from a variety of sources into a unified data mining warehouse. Based on the InterMine platform, MaizeMine provides an assortment of web-based search tools as well as an application programming interface (API) for programmatic access. The simple keyword search retrieves report pages for database objects such as genes and transcripts. Preconstructed template queries provide simple menus that enable users to perform complex queries integrating diverse data collections. The QueryBuilder can be used to modify template queries or construct custom queries. The List Tool takes an input list of identifiers that can be saved and used in further queries, gene set enrichment analysis and set operations such as union and intersection. The Regions Search Tool is used to retrieve selected genomic features based on chromosome coordinates. Query results are provided as tables that can be further filtered and exported in several formats, including tab-delimited, comma-separated, XML, JSON, GFF3, BED and fasta. The InterMine API libraries support programmatic access to template and custom queries with Perl, Ruby, Java and Python. We have recently developed API support for programmatic access to the MaizeMine Regions Search Tool using Python. Originally focused on the B73 reference genome, we expanded MaizeMine to encompass the maize pangenome based on B73 and 25 high quality Nested Associated Mapping (NAM) founder genome assemblies. Datasets included for each of the lines include Gene Ontology (GO) annotations, gene symbols and descriptions, and metabolic pathways using the E2P2 prediction pipeline. As the common parent to the NAM populations, the Zm-B73-REFERENCE-NAM-5.0 genome assembly continues to serve as the backbone for MaizeMine and is supported by a rich collection of supporting data sets which includes gene expression data from MaizeGDB's qTeller, SNPs from Ensembl Plants, pathways from Plant Reactome and KEGG, Gene Ontology and protein annotations from Uniprot, protein domains from InterPro, and a collection of maize community data sets. Here we highlight those community data sets which include Uniform Mu and Ac/Ds insertions, transcription start sites from multiple tissues, enhancer regions, and an atlas of genome wide association studies. We invite suggestions from the research community for datasets to include in future MaizeMine releases.

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P133

The prediction of Zea mays (maize) and Fusarium graminearum host-pathogen protein-protein interactions using fine-tuned protein language models and diffusion

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In silico methods of predicting protein-protein interactions (PPIs) that drive the plant immune response have been heavily studied in recent years to identify target genes for crop resistance. However, the PPI network between plant hosts and their pathogens is relatively unmapped, likely due to the limited data on known interspecies PPIs in current databases. The objective of this study was to overcome this obstacle by using recent advances in artificial intelligence, and predict interspecies PPIs between maize (Zea mays L.) and plant pathogenic fungi, Fusarium graminearum (ear/stalk rot disease). Firstly, candidate fungal effector proteins from F. graminearum were predicted using the computational biology tools EffectorP and SecretSanta. The binding residues of each effector protein were imputed using ESMBind, and RFdiffusion with Protein-MPNN were used to generate the structures of four potential proteinaceous binders. The binders were filtered by predicted Local Distance Difference Test (pLDDT), predicted Aligned Error (pAE), and root-mean-square deviation (r.m.s.d). Maize proteins which shared structural similarity to the binders were identified using FoldSeek. The interface of each presumptive PPI was then modeled in ColabFold, and the pair was retained if there was evidence for indirect/direct interactions between the two chain's residues. The maize proteins were enriched for Pfam domains (i.e., Snf7, SNARE, pectin methylesterase inhibitor) and gene ontology terms that indicate the maize binders participate in vesicle transport, membrane trafficking, and enzyme regulation. Although the PPIs need to be experimentally validated, this study presents the framework for generating predictions of interspecies PPIs that can be explored for virtually any plant or pathogen of interest. Understanding these interspecies PPI could aid the development of control strategies that reduce crop disease or identify target genes for developing crop resistance.

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P134

Identification and annotation of stress response transcriptional regulatory mechanisms in maize

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Maize (*Zea mays* ssp. mays) is one of the major crops cultivated worldwide for food, feed, and fuel. With the high demand for maize production, it becomes imperative to improve the stress tolerance strategies in maize as increases in climate variability can limit maize yield. MaizeGDB has curated genes related to stress response and climate adaptability using transcriptome approaches to enhance the functional annotation of traits related to biotic and abiotic stresses. Transcriptomic and epigenomic profiling has been used to explain the gene regulatory mechanisms in other plants during stress, but such integrated studies in maize during stress still need to be explored in depth**.** We aim to identify the interplay between gene expression and histone modification in maize under stress conditions, building upon our previous expression functional annotation analyses. We accessed publicly available ChIP-Seq datasets related to biotic and abiotic stress generated from tissues of the B73 cultivar. The high-quality ChIP-Seq reads were mapped to the most recent version of maize reference genome, B73v5. We will identify which genes are differentially associated with stress-response peaks, and correlate such genes with our previous stress-response functional analyses. Further downstream analysis will be performed, such as pathway enrichment and motif analysis. Our research will serve as a comprehensive resource for annotating maize stress genes.

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P135

Pan-gene and gene family data at MaizeGDB

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With increasing numbers of reference-quality genomes, especially the set of NAM founders and B73 reference assemblies, pan-genome data and research within *Zea mays* and the grasses becomes increasingly accessible, permitting expanded research across maize diversity and within grass species. The completion of 15 reference-quality genomes for *Zea* and related grass species provides a basis for calculation grass gene families, with a focus on species in the *Andropogoneae* tribe, to which *Zea mays* belongs. The Pandagma pipeline (https://github.com/legumeinfo/pandagma) was used for both the pan-gene and grass gene family analyses.
MaizeGDB released the initial version of its pan-gene data center in November, 2023.

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P136

A developmental series transcriptomic study of nitrogen-related heterosis

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Nitrogen use efficiency (NUE) has been documented to be increased in maize hybrids with both uptake and internal use phenotypes enhanced compared to inbred parents. Some traits involved in this are reliant on developmental phase-specific variations in organ nutrient remobilization such as stay-green, inspiring a season-wide assay of leaf-level gene regulation. B73, H99, and F1 hybrids of B73 and H99 were grown in paired N-deficient and -sufficient plots and sampled at early and mid-vegetative, and flowering timepoints for RNA-sequencing. H99 knockout mutants of a key nitrate transporter and F1 crosses of this mutant with B73 were grown and sampled in the same field in order to further dissect the role of nitrogen status signaling in heterosis NUE. Classical likelihood ratio testing reveals differential gene expression of expected nitrogen metabolism genes and indicates possible master regulators associated with their targets by gene network inference. With the quality of the pan-genome, SNP-aware alignment and RNA quantification also helps to piece apart the gene expression contribution of each parent and demystify some of the regulatory landscape.

Gene / Gene Models described: *nrt1.1b*; Zm00001eb023600
Funding acknowledgement: National Science Foundation (NSF)

P137

A multi-omics integrative network map of maize

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Networks are powerful tools to uncover functional roles of genes in phenotypic variation at a system-wide scale. Here, we constructed a maize network map that contains the genomic, transcriptomic, translatomic and proteomic networks across maize development. This map comprises over 2.8 million edges in more than 1,400 functional subnetworks, demonstrating an extensive network divergence of duplicated genes. We applied this map to identify factors regulating flowering time and identified 2,651 genes enriched in eight subnetworks. We validated the functions of 20 genes, including 18 with previously unknown connections to flowering time in maize. Furthermore, we uncovered a flowering pathway involving histone modification. The multi-omics integrative network map illustrates the principles of how molecular networks connect different types of genes and potential pathways to map a genome-wide functional landscape in maize, which should be applicable in a wide range of species.

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P138

A multi-organ maize metabolic model connects temperature stress with energy production and reducing power generation

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Climate change has severely affected maize productivity worldwide. To mitigate the issue, a holistic understanding of metabolic crosstalk among its organs is important. Thereby, we reconstructed the first multi-organ maize metabolic model, *i*ZMA6517, and contextualized it with heat and cold stress transcriptomics data using **EX**pression dis**T**ributed **REA**ction flux **M**easurement (EXTREAM) algorithm. Furthermore, implementing metabolic bottleneck analysis on contextualized models revealed differences between these stresses. While both stresses had reducing power bottlenecks, heat stress had additional energy generation bottlenecks. We also performed thermodynamic driving force analysis, revealing thermodynamics-reducing power-energy generation axis dictating the nature of temperature stress responses. Thus, a temperature-tolerant maize ideotype can be engineered by leveraging the proposed thermodynamics-reducing power-energy generation axis. We experimentally inoculated maize root with a beneficial mycorrhizal fungus, *Rhizophagus irregularis*, and as a proof-of-concept demonstrated its efficacy in alleviating temperature stress. Overall, this study will guide the engineering effort of temperature stress-tolerant maize ideotypes.

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P139 @JCallwoo

Advancing maize phenotyping through automation and data analysis

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Maize is pivotal in supporting global agriculture and addressing food security challenges. To better understand the genetic factors that underpin maize growth, quantitative phenotyping of traits is essential. Root systems are challenging to phenotype given their below-ground, soil-bound nature. In addition, manual annotations of root images are tedious and can lead to inaccuracies and inconsistencies between individuals, resulting in data discrepancies. To address these issues we have developed an automated phenotyping pipeline utilizing Root Painter , Rhizovision , and R for maize root image analysis and efficient extraction of phenotypic data. This pipeline was tested on images of field-grown maize crown root systems (stages V6-V8) from the Wisconsin Diversity panel. By minimizing user input and increasing automation, these tools improve the consistency and accuracy of data metrics. Root Painter, a segmentation application based on U-Net with a user-friendly interface, specializes in identifying roots and nodes. 123 images were annotated in RootPainter's interface for training. Resulting in precise differentiation between roots and non-root structures, enabling unsupervised crown root phenotyping. Finally, these segmented images were subsequently processed using Rhizovision's batch image processor, extracting numerous key root traits, including total root length, network area, and volume. The output from Rhizovision was then analyzed using an R script, incorporating statistical and visualization packages. Comparing the results obtained from our automated phenotyping pipeline with manually measured root systems demonstrated increased accuracy and consistency across researchers. This integrated pipeline saves user time and reduces costs by harnessing open-source maize phenotyping software and robust data analysis techniques.

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P140

Are the non-coding regions really non-coding? Large-scale identification of orphan genes from B73 v5 genome

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Orphan genes are young genes often generated by *de novo* gene birth from previously non-coding regions and encode proteins that have no homologs in other species. Orphan genes from diverse plant species have been experimentally demonstrated as essential for nutrient utilization, stress tolerance, or disease resistance. Orphan genes are often short in length and mono-exonic, and lack the canonical features of mature conserved genes. Hence, they are largely ignored by many gene prediction approaches. Using BIND, a gene prediction pipeline that combines *ab initio* prediction and evidence-based prediction utilizing ~1,500 publicly available maize B73 RNA-seq datasets from diverse biological conditions, we have identified ~38,000 transcripts that potentially encode orphan proteins. About 88% of these transcripts lie within genomic regions previously considered to be intergenic, and the remaining 12% are likely the alternative splicing products of mature genes. Approximately 1,500 orphan transcripts have translation evidence supported by analysis of 175 publicly available Ribo-seq datasets that contains only a subset of conditions of the RNA-seq datasets used to identify these orphan transcripts and thereby represents a lower bound. Three tools to evaluate coding-potential were employed to analyze the orphan transcripts, and ~39% are predicted to be protein-coding. Due to the lack of homology with other species, identifying functions of orphan genes is a major challenge. As an example strategy, we present a joint statistical analysis between the maize silk orphan transcriptome and cuticular wax metabolome. This analysis identifies ten orphans with translation evidence that are significantly associated with cuticular wax composition, thus providing novel directions for characterizing orphan function. Collectively, by identifying orphan transcripts expressing under specific conditions, this study demonstrates the complex and dynamic nature of the maize transcriptome and provides rich resources for further exploration of maize genome biology and its impact on crop phenotypes.

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P141

BRIDGEcereal webapp for survey and graph indel-based haplotypes from pan-genomes

(submitted by Xianran Li <xianran.li@usda.gov>)
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One of key reasons to develop a pan-genome is to bolster the process of identifying and characterizing causal polymorphisms for genes underlying phenotypic variations. Literature reviews show that large insertion and deletion (indel) polymorphism is an important category of casual polymorphisms, however, the process of identifying large indels from a pan-genome is challenging. To overcome this challenge, we first developed two unsupervised machine learning algorithms, CHOICE (Clustering HSPs for Ortholog Identification via Coordinates and Equivalence) and CLIPS (Clustering via Large-Indel Permuted Slopes), then constructed an interactive webapp BRIDGEcereal (https://bridgecereal.scinet.usda.gov/) to expedite this process. Over 120 assemblies, including 40 maize inbred genomes and other cereal crops, were compiled into the database. Through the intuitive graphical user interphase, the only required input is a gene model ID to mine through publicly available pan-genome for surveying and graphing indel-based haplotype.

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P142

Benchmarking across-species RNA expression prediction within maize and its wild relatives

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Targeted genome editing and selection technologies have the potential to accelerate plant breeding pipelines in any species but require prior knowledge of what changes to make and where to make them. One promising approach for proposing effectual edits is by querying deep learning models trained to predict cellular features such as RNA expression from DNA input. Deep learning models benefit from large and diverse training sets of different tissues and genotypes, which are rarely available within a single species. To train RNA expression models on larger sample sizes, we leveraged new long-read genomes and RNA-seq data from 15 wild species closely related to maize, sorghum, and sugarcane. Millions of years of evolution across these species has resulted in a large, diverse pool of training alleles. As model input, we extracted 1,024 base pairs upstream of each gene's translation start site. This sequence data was used to train model architectures shown to be state-of-the-art on human data. Testing the trained models on NAM parent expression data shows Spearman correlations between 0.45 and 0.55. All architectures fail to accurately rank NAM alleles by expression, showing an average Spearman correlation within a pan-gene of less than 0.10. This suggests that smaller state-of-the-art supervised models are neither able to generalize well across related species nor sensitively separate alleles within species, the latter of which agrees with recent work within humans. Large language models have demonstrated success in answering questions and modeling proteins but have so far been evaluated only on species in the training set for RNA expression tasks. A model that successfully learns gene regulation should be able to generalize to unseen related species. Therefore, we are releasing the dataset and codebase for this work as a community benchmark to evaluate new architectures on our across-species and across-allele tasks.

Funding acknowledgement: United States Department of Agriculture (USDA)

P143

Breeders' genomic hub: Integrating analysis tools with data

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With increasing amounts of genomic data, the demand for fast, reproducible, and robust bioinformatic pipelines is paramount. The Breeders' Genomics Hub, built on a JupyterHub framework, integrates a suite of open-source tools for collecting, analyzing, and predicting biological patterns from phenotypic and genotypic data. This unified platform not only streamlines data analysis but also simplifies pipeline documentation. Utilization of Jupyter Notebooks within the hub promotes sharing and reproducibility.
One of the strengths of the Breeders' Genomics Hub is it provides researchers with access to powerful, yet user-friendly, data sources and software tools. It leverages the Breeding Application Programming Interface (BrAPI) web services to retrieve data from common breeding databases using BreeDBase, BMS, or Gigwa. For instance, rTASSEL and rPHG, accessible via a R programming kernel in Jupyter Notebooks, connect users to TASSEL and the Practical Haplotype Graph (PHG). PHG is a graph-based tool for haplotype calling from high-throughput sequencing data. BioKotlin, a bioinformatics library offering the performance of compiled languages (>50-fold performance) with the ease of Python-like scripting, is also available on the Hub. Researchers can harness these tools to retrieve PHG data, integrate and preprocess it using rTASSEL and BioKotlin, visualize it with standard libraries, and employ machine learning or R models for insightful genomic predictions.
By using the Breeders' Genomics Hub to link data from multiple sources and perform advanced analysis plant breeders can gain valuable insights into the relationships between genotype and phenotype and make informed breeding decisions. Ultimately, the Breeders' Genomics Hub not only fosters collaboration among scientists but also enables integration with genomic tools, offering a centralized repository for data pipelines and results.

Funding acknowledgement: National Science Foundation (NSF), United States Department of Agriculture (USDA), Cornell University, USAID, Bill and Melinda Gates Foundation

P144

Brobot & RootTaggingGui: An image acquisition and processing pipeline for high-volume phenotyping

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Collecting brace root phenotyping data is instrumental to understanding the development and structural contributions of brace roots in maize and sorghum. Phenotypes such as brace root width, whorl count, emerged brace root count per whorl, and geometric attributes of stalk-to-ground architecture are extremely difficult and time-consuming to collect manually in the field. To efficiently collect this data at a large scale, we have developed the Brace Root Robot, named Brobot (bˈrəʊbɒt), a remote-controlled sand-crawler droid that takes high-fidelity images of brace roots in the field as it is piloted between rows. To extract the phenotyping features from these images, we have developed RootTaggingGUI to easily measure attributes of brace root architecture using pixel distances. In this poster, we discuss the accuracy and efficiency of the current implementation, as well as the improvements planned for the future, including a development path to autonomous driving of the Brobot and autonomous phenotypic feature extraction.

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P145 @hwb333

Chromatin structure and particle mapping in maize root tip nuclei using MNase digestion assays.

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The chromatin structure of maize root nuclei was examined by analyzing nuclease sensitivity and cleavage patterns from the replication-active terminal 1 mm of the maize seedling root tip. We developed several related assays, including DNS-seq and MOA-seq, utilizing micrococcal nuclease (MNase) as a probe for chromatin structural features. These features are coupled to gene regulation, phenotypic traits, and cistrome occupancy. DNS-seq and MOA-seq generate NGS libraries from small DNA fragments isolated from formaldehyde-fixed chromatin, employing both defined and variable concentrations of MNase. We analyzed the terminal 1 mm of seedling root tip chromatin in conjunction with two projects: one aiming to map open chromatin (NSF IOS 1444532) and the other studying DNA replication timing (NSF IOS 2025811). Our data tracks are shared on MaizeGDB and via our UCSC genome browser on genomaize.org. DNA replication time profiling provides an orthogonal measure of chromatin status, ideal for integrative and comparative epigenomics. Here, we summarize the assays and data analysis pipelines for visualizing, mapping, and interpreting coverage data of MNase-produced DNA fragment centers (frenters) from maize root tip samples. The MNase coverage profiles facilitate peak calling to: (1) map nucleosome occupancy from DNS-seq heavy digest frenters, (2) identify large hyper-sensitive footprints revealing nucleosome-sized particles or large complexes from DNS-seq light digest large fragment frenters, (3) locate small hyper-sensitive footprints indicating transcription factor (TF) occupancy candidates for cis-element discovery and functional analyses from DNS-seq or MOA-seq, and (4) identify regions of potential false positives using chromatin-free purified DNA in MOA-seq-like digests. Collectively, these findings demonstrate how MNase digestion assays are useful for defining chromatin structural features at the level of DNA-protein particle locations, including nucleosomes and DNA-bound transcription or replication factors, thereby enhancing our understanding of epigenomic states and genetic processes.

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P146

Chromosome-level assembly and comprehensive annotation of the W22 and PH207 maize inbreds

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Advances in genomic technologies have paved the way for high-quality de novo assembly of complex genomes, enabling researchers to unravel the intricacies of genetic architecture. This study presents the assembled and annotated genomes of two key maize inbreds, W22 and PH207. The W22 genome, historically recognized for its pivotal role in maize genetics, has served as a foundational resource for studies of paramutation. PH207, belonging to the Iodent germplasm, is a crucial asset for the production of temperate commercial maize lines and has played a significant role in heterotic hybrid development, shaping modern maize breeding programs. Leveraging PacBio HiFi long reads and Bionano optical maps, we achieved high-quality assemblies for both W22 and PH207. W22 exhibited a contig N50 of 135.8 Mb and an L50 of 7, comprising a total of 102 contigs. PH207 displayed a contig N50 of 73.6 Mb and an L50 of 9, consisting of 121 contigs. The final pseudomolecule assemblies were generated using pan-genome anchor markers. Our annotation strategy involves utilizing a diverse set of RNA-seq datasets, incorporating Cap Analysis of Gene Expression (CAGE) data for the precise identification and annotation of untranslated regions (UTRs). Additionally, Iso-Seq long reads will be employed to uncover full-length isoforms. We aim to comprehensively characterize the genomic composition by comparing the assemblies to existing maize inbred lines and fully characterizing the repeat elements in the genomes. The resulting assemblies and annotations, supported by genomic browser integration, will be publicly available on MaizeGDB. This detailed understanding of the genomic structure and content of W22 and PH207 will serve as invaluable resources for the maize research community, providing a foundation for further investigations into maize genetics, breeding, and functional genomics.

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P147

Comparison of rRNA depletion methods in maize

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To assess how transcription, particularly the rate of transcription, correlates to maize root tip DNA replication timing, we made RNA-seq libraries from nascent, nuclear, and cellular RNA isolated from the 0-1 mm terminal portion of maize root tips. To obtain a more inclusive representation of the transcriptomes for mRNAs, non-polyA RNAs, antisense transcripts and long non-coding (lnc) RNAs in the RNA-seq libraries, we used ribosomal RNA (rRNA) depletion instead of polyA selection prior to library construction. We compared commercially available kits (Invitrogen RiboMinus, Vazyme Ribo-off, and Qiagen QiaFastSelect) for plants that use probe hybridization to rRNAs. To analyze the ribo-depletion efficiencies of the kits and to filter out highly repetitive transcripts like tRNAs and signal recognition particle (7SL/SRP) RNAs in the resulting RNA-seq data, we generated maize-specific, custom databases of 5S, 5.8S, 18S, 28S, internal transcribed spacer (ITS), mitochondrial and chloroplast rRNAs, tRNAs and SRP sequences to use with the SortMeRNA bioinformatic tool. All kits reduced the amount of rRNA, but none successfully removed all rRNAs. With the Vazyme kit, the type of RNA used for the library impacted the amount of rRNA contamination in the reads, with 10%, 30%, and 80% rRNA contaminants in the nascent, nuclear and cellular RNA-seq reads, respectively. In nuclear RNA-seq libraries, ~30% of the RNA-seq reads corresponded to rRNA sequences when either the Vazyme or the Qiagen kit was used. However, the kits differed in the types of rRNA that were not removed efficiently. The major read contaminant for the Vazyme kit was from 28S rRNA (89% of the reads removed by SortMeRNA databases), whereas for the Qiagen kit the major contaminants were from mitochondrial and ITS rRNAs (~60% and 27% of SortMeRNA reads, respectively). Further characterization of the kits and a description of how the maize rRNA databases were generated will be presented.

Funding acknowledgement: National Science Foundation (NSF)

P148 @ADesbiezPiat

Coupling ecophysiology and genetics in a digital environment for modeling crop adaptation

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Adapting maize to climate change encompasses a three-way interaction between genetic variation, environmental plasticity, and directed evolution. Here, we show how interfacing digital and life sciences offers new possibilities to model more realistic genotype-to-phenotype relations in evolving populations, to build the underpinnings of a new integrative synthesis, and guide progress in plant breeding. In a novel virtual system that couples evolutionary genetic models (EGM) with crop growth models (CGM), we bridge a divide between models (and modelers) that: [i] simulate daily growth across a plants life cycle leading to phenotypes for emergent traits such as flowering time and yield across environments (physiologists, agronomists); and [ii] how selection can act on those outcomes to drive evolution and impact diversity (geneticists, breeders). As a proof-of-concept, we focus on flowering time, using a diversity panel in a high-throughput phenotyping plateform as a training population to incorporate the genetic architecture of flowering trait components into the EGM. New genetic parameters of virtual recombinants (outputs of the EGM) are then passed to the CGM to predict the flowering dates of recombinant inbred lines in different environments based on their genetic makeup, and those predictions are then compared to observed data. Our system highlights the need for specifically designed experiments to accurately estimate GxE interactions. We further investigate how 20 years of climate change is impacting maize flowering time in terms of variance components, leading to compressed variation in flowering time with potential impacts on the selection response. In turn, we explore how interactions between genetic architecture, recombination and parental sampling can determine the adaptive potential of maize to different environments under climate change.

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P149 @jefowlerjr

EarVision.v2 detects spatial effects of pollen mutations on kernel distribution across the maize ear

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Male gametophytes transport sperm cells from the anther to the ovule via pollen grain and pollen tube, reaching the embryo sac for double fertilization. The haploid nature of the gametophyte results in more direct expression of phenotype from mutation than in a diploid, with potential for subtle indirect effects on the final outcome: generation of a seed. For example, the architecture of the maize ear, with ovules at the base vs apex associated with silks of different lengths, raises the possibility that certain pollen mutants are associated with non-random effects on the spatial distribution of successful fertilization. In order to address this hypothesis across a large set of mutants, we have updated the EarVision computer vision pipeline, which identifies GFP-marked mutant and wild-type kernels from ear images, to both enhance its effectiveness and to output spatial information regarding kernel distribution. EarVision.v2 incorporates an ear scanner engineered for higher throughput, improved kernel classification across imaging conditions, and automated recognition of images that are unsuitable for computer vision phenotyping. In addition, it generates ear map files for each image, associating kernel phenotypes with specific spatial coordinates for downstream analysis. An R pipeline divides each map file into spatially distinct bins along the length of the ear, and assesses whether mutant transmission rates vary significantly across these bins. Our initial analysis of >80 GFP-marked mutant alleles has identified at least three alleles associated with non-random spatial outcomes. For example, the *tdsgR102H01* insertion, which interrupts a putative actin-binding protein, conditions a reproducible apex-to-base gradient of decreasing transmission success for mutant pollen, consistent with impairment of pollen tube growth in the mutant. Expanding our analysis to additional pollen mutants should allow us to discern overall trends - e.g., how often are changes in pollen fitness associated with spatially-influenced outcomes?

Gene / Gene Models described: ; Zm00001eb283600
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P150 @6zongyan

Enhancing plant genome annotation through DNA language model: A focus on arabidopsis and maize

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Advancements in genome sequencing have revolutionized the study of diverse organisms by making it more affordable and accessible. Despite these strides, deciphering the intricacies of genomic structural and functional variation remains a significant challenge. Genome annotation is the process of inferring and modeling transcription and translation across the genome. Genome size, complexity, and diversity further complicate the annotation process. To enhance the speed and accuracy of gene annotation in plant genomes, we fine-tune a machine learning model called the Genomic Pre-trained Network (GPN), which could help us translate the DNA language model. This method streamlines the gene prediction process, enabling the recognition of small peptides often overlooked by traditional tools. Our approach uses Ribosome profiling (Ribo-seq) data from Arabidopsis to scan the genome and label the gene boundaries of the potential translation initiation sites (TIS) and stop sites. Applying GPN embeddings to a trial dataset containing 1.1 million potential initiation sites and 3.6 million potential stop sites in Arabidopsis showed a prediction accuracy of 92% in identifying the putative initiation and stop sites. This accuracy enables the precise location of genes and facilitates the identification of splice donor and acceptor sites. Finally, to validate the efficacy of the GPN in maize, we compared our model with existing gene prediction tools. Our findings showcase the effectiveness of a DNA language model as a valuable tool and resource for the plant research community. This work provides a robust solution for genome annotation challenges and significantly advances our understanding of plant biology.

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

P151

Ensembles of deep learning, machine learning, and linear models outperform individual models for maize yield prediction in diverse environments

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Improving phenotypic prediction is necessary for more effective crop improvement and forecasting. This is especially true for crops which are to be grown in diverse environments and for traits with substantial gene-by-environment effects. Considerable thought has been devoted to improving phenotypic prediction by seeking to identify the best modeling approach including linear predictors, machine learning, and deep learning models. Here we explore a complementary approach to improve accuracy by using predictions from multiple models together in an ensemble. We demonstrate that an ensemble optimized by a genetic algorithm can reduce error by 6.98% relative to the best non-ensembled model. The optimal ensemble doesn't consist only of the most accurate models but leverages lower performing models as well. This technique is effective even without significant optimization. In simple ensembles consisting only of two models we report an improvement relative to the base models in 76.76% of cases. While future advances in modeling approaches for phenotypic prediction are of key importance, this work demonstrates that ensembling can robustly increase the value provided by existing models.

Funding acknowledgement: United States Department of Agriculture (USDA)

P152

Genetic determinants of Northern Corn Leaf Blight resistance revealed by multi-scale systems genetics

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Quantifying changes in transcript and protein expression in response to a specific biological process has been essential for the discovery of genetic determinants controlling the expression levels of gene-products. However, the role of post-translational modifications, such as phosphorylation, has not been explored on such a high-throughput scale. Here, we integrated transcriptome, proteome, and phosphoproteome measurements to map molecular quantitative trait loci (QTL) and build predictive regulatory networks from Mo17xB73 derived recombinant inbred line leaf tissue infected with Northern Corn Leaf Blight (NLB). We detected 34,816 transcripts, 11,618 proteins, and 42,078 phosphosites and further mapped 34,056 transcript, 5,321 protein, 4,935 phosphosite, and 11,393 metabolite QTL. Novel genetic determinants of NLB resistance were validated using CRISPR/cas loss-of-function lines. Our results demonstrate the feasibility of high-throughput mapping of multi-level molecular QTL and identify novel genes required for resistance to NLB.

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P153

Genomic analysis of stability in grain composition in maize

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Grain composition in maize grain plays a critical role in ensuring nutritious food supply and a variety of products that are important to the sustainability of human societies. As growing conditions become increasingly unpredictable due to rapid climate change, understanding the genetic basis of stability in grain composition is imperative. Despite previous research on the genetic and environmental factors influencing grain composition, the interaction between genotype and environment (GEI) remains underexplored. To address this, we conducted a comprehensive GEI analysis on over 500 maize inbred lines grown in five environments, for compositional traits including protein, starch, fiber, ash, moisture, and fat. In addition, we derived latent phenotypes to represent composition as a single holistic metric, and reduced bias due to genetic correlations. We assessed trait stability using a combination of parametric and nonparametric approaches that included Finlay-Wilkinson regression, AMMI-based models, and BLUPs. Our analysis incorporated a combination of single nucleotide polymorphisms (SNPs) and structural variants, to investigate the genetic architecture of stability in composition using single and multi-trait genome-wide association studies (GWAS). In this poster, we present a summary of the genomic markers found to be associated with stability for single traits as well as with latent phenotypes representing composition as a whole. The findings from this study provide valuable insights into the genetic mechanisms underlying stability in grain composition, paving the way for the development of adaptable maize cultivars with proper grain quality.

P154

Gramene PanMaize: One-stop pan-genome browser for exploring the rich genetic diversity in maize

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The Gramene project (http://www.gramene.org) played a crucial role in sequencing the first maize reference, B73, and more recently, the 25 NAM founders. The project remains actively involved with the maize community. Expanding on the Gramene and Ensembl infrastructures, the project developed four pan-genome subsites dedicated to individual crop groups: maize, rice, sorghum, and grape. In 2021, the maize pansite (https://maize-pangenome.gramene.org) was launched, hosting 37 maize genome assemblies, including three versions of the B73 reference, Ab10, 10 NAM genomes, and four European flint genomes. Each maize accession has a separate genome browser, facilitating gene-based views through text-based searches or BLAST. B73 serves as the reference assembly, anchoring gene expression, population, and pathway views. The site enables detailed exploration of transcript abundance across tissues and developmental stages, aiding in paralog expression analysis. Phylogenetic analyses rely on maize-centric gene trees, forming the basis for synteny maps and supporting rapid traversal between maize accessions and other species. The gene-centric views offer insights into allelic genes within and across species, gene expression, variation data, and lineage-specific expansions and contractions. Protein homology is visualized through amino acid alignments and gene neighborhood conservation. Community curation tools are deployed from the homology tab, allowing users to flag potential structural annotation issues. Recent work utilized gene trees to build a pan-gene index, enhancing gene structural annotation workflows. SNPs are easily viewed and filtered based on predicted functional effects and impact, downloadable as images and tables. Release 4 will include the genome teosinte inbred TIL11 and histone modification and transcription factor ChIP-seq data in parallel with transcriptomics datasets in five different tissues. In line with FAIR principles, standard identifiers or rsIDs will be assigned to SNP variants. The project continues to collaborate with the community for data stewardship, training, and feedback. Funded by USDA-ARS-8062-21000-041-00D.

P155 @KatieMurphyPhD

High-throughput phenotyping of maize stress responses in controlled environments

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Understanding maize responses to environmental stressors is critical to ensuring a sustainable food supply in the face of climate change. Here, we first used high-throughput, image-based phenotyping to characterize the drought response of 46 inbred maize varieties. PlantCV, an open-source image analysis platform, was used to assess plant growth and color phenotypes and determine which varieties were most susceptible to stress. Next, we used weight-based phenotyping and the DiTech PlantArray system to demonstrate that varieties with more severe drought stress phenotypes have a higher normalized transpiration rate. The facilities and services shown here are available to both internal and external users at the Phenotyping Core Facility in the Donald Danforth Plant Science Center for high-throughput assessments of plant phenotypes under controlled environmental conditions.

P156

Identification of cold-adapted proteins in Poaceae using machine learning and comparative genomics

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The rapidly growing repository of plant genomes is opening new avenues in comparative genomics for gene discovery. This advancement is pivotal in the race to identify novel genetics in agricultural crops to mitigate climate change. This study seeks to leverage the evolutionary diversity in the Poaceae family to detect candidate genes that can enhance cold tolerance in maize (Zea mays). Cold tolerance would allow growers to plant maize earlier, avoiding late season heat risk and reducing greenhouse gas emissions from the soil. Initially, we assemble homologous proteins across species within the grass family using sequence alignment and phylogenetic analysis. A protein language model (pLM) then encodes the amino acid sequences into mathematical representations known as embeddings, which encapsulate their evolutionary, structural and functional characteristics. Finally, the embeddings are used to train a supervised machine learning model to predict the likelihood of a protein descending from a cold- or warm-adapted species. We expect that proteins classified as cold-adapted present promising candidates for introgression into warm-adapted crops, such as maize, to improve resilience and offset greenhouse gas emissions. Next steps will focus on validating candidate proteins via transcriptomic analysis and gene editing. Our focus on the Poaceae family not only aims to provide insight into the genetic mechanisms of cold tolerance but also to serve as a model for gene discovery for practical applications in other crops.

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P157

Impacts of structural variants on accurate SNP imputation

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Genotype imputation is a valuable statistical tool used to infer missing genetic markers, enhancing the mapping resolution and power of genome-wide association studies (GWAS). The imputation of genetic markers are usually done by analyzing linkage disequilibrium (LD) patterns and haplotype information in a population. However, genotype imputation is not always accurate. In this study, we examine the accuracy of imputing missing SNP data based on the marker information of 509 maize inbred lines, which are part of the Wisconsin Diversity Panel. SNP and SV marker information for this study were obtained from the short-read whole genome resequencing data of the inbred lines. Specifically, we are focusing on the errors in imputation that occur due to structural variants (SVs). The imputation of missing SNP markers will be done using the IMPUTE2 program. Error in the imputation of SNP markers within known deletions specific to each genotype will be assessed. The false imputation of SNP markers within deleted regions could impact downstream power to identify marker-trait associations, which will be tested in future GWAS.

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P158

Improving polyploid and heterozygous genome interpretation using hidden Markov models

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Genetic modification relies on identifying desirable genes within a related species. These relatives have historically been understudied for maize, with minimal genetic and genomic resources. Whole genome duplication events and hybridization have resulted in complicated polyploid genomes. This makes it difficult to both track and genotype genetic traits, and sequence and assemble their genomes. Recent decreases in the cost of sequencing and advances in long-read sequencing technologies have reduced these barriers for polyploids. These long reads can span tens of thousands of base pairs in length, and when assembling these reads into contigs, they often can reach highly repetitive regions that lead to assembly errors. In autopolyploids with low differentiation between homologous subgenomes, such collapse can even occur across both alleles and subgenomes. We developed a Hidden Markov Model (HMM) that uses the gene counts and read depths to assign the DNA sequence into two states, collapsed regions and separately assembled regions. This will improve the utility of these polyploid genomes when mapping RNAseq or functional genomic data, as coverage and counts can be adjusted by assembly depths. We also anticipate this approach may also improve the interpretation of highly heterozygous genomes, such as outcrossing maize.

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P159

K-mer trait association mapping and prioritization of candidate genes with Homotools for structural comparison

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Single nucleotide polymorphism (SNP) is the most common genetic variant used in Genome-Wide Association Studies (GWAS) to explore genetic controls of phenotypic traits. Alternatively, GWAS can use counts of substrings of length k from longer sequencing reads, k-mers, as genotyping data. Using multiple maize traits, namely, cob color, kernel color, kernel oil, and leaf angle, we demonstrated that k-mer GWAS can effectively identify trait-associated k-mers derived from polymorphic genomic sequences of causal genes. The polymorphisms include a wide range of genomic variation, including SNP, insertion and deletion, copy number variation, and translocation. Our study also showed that the k-mer can play a bridging role in integrating omics data for identification of candidate genes conferring a trait phenotype. To enable the exploration of genomic variation among alleles of candidate genes, we developed a command-line package called Homotools, which was further utilized to create a user-friendly online tool **http://tinyurl.com/maizegenomeHM**. In this poster, we show the combination of k-mer GWAS and Homotools is effective to accelerate functional genomics studies.

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P160

Life, the universe, and everything for $42: WideSeq mapping of maize mutants

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Convenient and economical genotyping methods and simplified bioinformatic workflows are critical for genetic studies and breeding. The declining cost of short-read sequencing, innovative library preparation methods, and multiplexing have enabled low-cost, large-scale genetic and genomic studies. Here, we present an uncomplicated method for whole-genome skim sequencing and PCR amplicon sequencing with bioinformatics pipelines sufficient for various genotyping and gene expression applications. The method employed at Purdue, nicknamed WideSeq, costs $21 (USD) and provides ~100k paired-end reads per sample when performed at scale. Input samples for WideSeq can be genomic DNA samples or double-stranded PCR products. The lower data throughput from WideSeq permits bioinformatic analysis on a regular laptop or desktop, which can aid in bioinformatic training and introduction to genomic analysis for students and first-time genomicists. Using whole-genome maize DNA samples derived from multiple pedigreed populations, including advanced backcrossed progenies, mapping of dominant and recessive mutants, bi-parental populations, near-isogenic lines, and recombinant inbred lines, we demonstrate that this genotyping method can map loci, detect donor introgressions, identify haplotypes, and perform allele-specific expression. Mutants can be mapped as single introgressions for $21 or by comparing mutant and wild-type siblings for $42. Interestingly, we can even perform mapping-by-sequencing to detect donor genomes derived from parents of unknown origin and map classical mutants of unknown pedigree without prior marker discovery. Location can be further refined following haplotype analysis and re-calculation of allele frequencies. Further cost reduction in WideSeq could be achieved by implementing this strategy at scale and optimizing the data throughput sufficient for a specific experiment. This approach can be implemented using commercially available sequencing services at a moderate cost increase.

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

P161

Mapping genomic elements in response to heat stress in maize

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Global warming is reshaping the crop growth environments and requires us to better understand plant responses to the rising temperature. As one consequence of global warming, heat stress can impact crop growth, development as well as the loss of grain yield. In the molecular level, heat stress can trigger gene activations in plants but molecular mechanisms remain unknown. Identifying genomic elements that are involved in the response process among varieties in one species can facilitate the understanding of potential genomic causal factors. We used maize as a model species to collect transcriptomic data in >100 inbreds and MNase-defined cistrome-Occupancy Analysis (MOA-seq) from B73 to understand heat response in maize. By developing a new eQTL mapping approach, we inferred hundreds of cis-regulatory elements (CREs) can be involved in gene regulation during heat stress and validated functions of selected CREs. This study provides insights on characterizing CRE functions in plant genome.

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P162 @NikeeShrestha2

Mapping loci controlling sorghum seed color using computer vision models pre-trained in distantly related grain crops

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Bioactive compounds like tannins, polyphenols, and other antioxidants are becoming more popular, and sorghum has a significant diversity of these compounds, represented by different seed colors. Computer vision models have the potential to enable rapid, accurate measurements of seed color with lower cost and human labor input. However, to date, the majority of published applications of these models have relied on building and training models for specific crop species, requiring both large ground truth datasets and substantial computational and machine-learning expertise from the researcher. Here, we evaluated the potential of transferring previously trained and published models for rice and wheat grain phenotyping to new applications in a new crop (sorghum). The model trained on rice seeds was selected based on seed segmentation accuracy in sorghum and used to segment scans of sorghum seeds from 1664 unique plots from a replicated sorghum diversity panel field experiment. Three seed morphology and six color-related traits were scored from the segmented scans. GWAS for seed morphology traits, conducted using a subset of sorghum genotypes for which whole genome resequencing data was available, identified 23 trait-associated SNPs, a number of which corresponded to previously described QTLs for sorghum seed size. GWAS for seed color phenotypes identified two known large-effect loci for seed color in sorghum (y1 and tan1) as well as an independently segregating large-effect seed color locus near the y1 locus, which exhibited significantly non-additive phenotypic interactions with y1. The effect of the newly identified locus was validated via genotyping a set of 96 additional sorghum genotypes not included in the original GWAS study. These results illustrate the potential and challenges of transferring computer vision-based grain phenotyping from proof of concept studies to application in biological question-focused research.

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P163

Microbial Partner (MiPner) analysis for the discovery and study of microbe-microbe interactions

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Although a few species of bacteria have been studied in great detail, little is known about the characteristics of the enormous number of microbe-microbe interactions that occur on a daily basis in almost every environment worldwide. We have developed a simple and robust technique to set up the foundation for investigating pairwise bacterial-bacterial interactions, using cell-cell binding to identify possibly functional bacterial species pairs. We first isolated, sequenced and annotated a *Serratia marcescens* strain (SMC43) from Georgia soil to use as "bait". Bacteria were then purified by their specificity in binding SMC43 that were attached to a wooden applicator stick. The microbe-saturated stick was streaked onto a plate containing bacterial media, and a few individual colonies were purified, while the whole stick-bound community was also examined by lifting the entire microbial growth off the plate as a mixture, and shotgun sequencing the DNA from this mixture. The isolated Microbial Partners (MiPners) were greatly enriched for previously undiscovered bacterial species, especially including members of the genera *Sphingobium* and *Caulobacter*, many novel. Some of the MiPners were unable to grow on the plate type tested in the absence of SMC43, suggesting an obligate partnership for those MiPner bacteria.

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P164 @vla_torres

Population level gene expression can repeatedly link genes to functions in maize

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Transcriptome-Wide Association Studies (TWAS) have the potential to provide single gene resolution identification of candidate genes in plants and serve as a complement to Genome-Wide Association Studies (GWAS). However, success in plant TWAS to date has been mixed. We generated a large expression dataset from 693 maize genotypes, measured in a common field experiment, and sampled over a two-hour period to minimize diurnal and environmental effects and using the full-length RNA-seq to maximize the accurate estimation of transcript abundance. TWAS analysis linked roughly ten times as many genes to variation in flowering time as GWAS conducted with the same trait data in the same population. The genes identified by TWAS included known true positive flowering time genes missed by GWAS, genes previously linked to flowering time variation in rice or Arabidopsis, and a modest number of genes without known links to flowering time but with plausible potential mechanisms. TWAS conducted using mature leaf tissue identified known true positive flowering time genes that act in the shoot apical meristem, and flowering time data from new environments beyond the one in which gene expression was profiled enabled the identification of additional flowering time genes. eQTL analysis of TWAS-tagged genes identified at least one additional known maize flowering time gene through trans-eQTL interactions. Collectively these results suggest the gene expression resource we have created can be reused to link genes to functions in determining variation in a range of plant phenotypes scored in diverse environments and expressed in diverse tissue types.

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P165 @tross_michael

Predicting cell type specific expression of maize gene models using DNA sequence

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Studies have demonstrated that transcriptional heterogeneity within and among cell types contained within the same organ is biologically important but is not captured by sequencing of pooled RNA samples extracted from bulk tissue samples. RNA-seq analysis via single cell sequencing has gained popularity in recent years due to its ability to paint a more detailed picture of variation in gene transcription, revealing complex genetic relationships in determining phenotypes in plants. However, the ability to utilize single cell RNA-seq may be hindered by the high cost and specialized technical expertise required to generate such datasets, resistance of tissues to treatment necessary for single cell sequencing and for some organs or species, the difficulty of accessing samples. We evaluated the potential of training machine learning based models to predict cell type specific expression patterns of genes from information on the DNA sequence of the gene and surrounding regions. We trained a custom built transformer model to predict cell specific expression of maize gene models using published single cell RNA-seq data from maize root tissues including nineteen labeled cell types. When trained on sequence data starting 2,000 base pairs upstream of the transcription start site and extending 500 base pairs downstream of the transcription stop site, this transformer model was able to predict the expression of individual gene models across 19 cell types with accuracies that exceeded controls. Results show that the transformer model is able to predict cell type specific expression of gene models using their genomic sequence while also illustrating the importance of employing suitable benchmarks for evaluating the performance of ML-based predictions in the maize genome.

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P166

Prioritizing deleterious mutations in Maize by DNA language model

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Modern maize breeding has significantly benefited from heterosis, where hybrids exhibit superior fitness traits, such as yield, compared to their parental inbred lines. A key aspect of this phenomenon is the role of deleterious mutations in reducing the fitness of inbred lines, highlighting the importance of deleterious mutation detection for Maize breeding improvement. Leveraging advancements in deep learning and natural language processing, we developed a convolutional DNA language model to prioritize deleterious mutations across the maize genome. This model treats each nucleotide as a 'word' in a self-supervised learning framework, akin to techniques used in natural language processing. Trained on genomic data from nine Poaceae species, including maize, spanning an evolutionary timeline of 49 million years, our model uncovered deep conservation patterns within the Poaceae family. After pre-training, the model was fine-tuned to predict conservation of both coding and non-coding SNPs, achieving accuracies of 0.82 and 0.95, respectively. Application of the pre-trained model to Maize haplotype 3 revealed that predicted deleterious mutations are enriched with rare alleles more than threefold compared to baseline. These findings demonstrate the effectiveness of our DNA language model in identifying deleterious mutations, offering substantial potential to enhance maize hybrid breeding by enabling more precise masking of predicted deleterious mutations.

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P167

Remote sensing for response to nitrogen fertilizer in maize

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Modern maize hybrids prolong the period that they photosynthesize and accumulate Nitrogen (N) out of the soil which has helped them produce more yield per unit of N fertilizer. However, the increase in post flowering activity is inversely correlated with N remobilization from the leaves. Further gains in N response could be achieved by breaking this association, but doing so requires an in-depth understanding of the temporal dynamics of maize canopy traits and plant N mobilization. Leaf nutrient samples were collected at five time points and remote sensing phenotypes were extracted from Unoccupied Aerial System (UAS) imagery (orthomosiacs and point clouds). Spectral indices and point-cloud based metrics were used to investigate the relationship between changes in N storage dynamics and yield among hybrids grown in low and high N treatments. From these combined phenotypes, it is possible to dissect how rate of growth and canopy health help to describe hybrid response N and also provide clues for how to break the negative relationship between yield and N remobilization.

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P168 @GrameneDatabase

Swift pan-genomic methods for comprehensive genome annotation in maize genomes

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The advancement in generating high-quality genome assemblies from long reads has facilitated standard practices, yet precise gene structure annotation remains a formidable task. This challenge persists due to algorithmic predictiveness and inconsistent transcriptome evidence. A singular reference annotation inadequately represents a species' coding potential, while de novo annotations face sensitivity and specificity issues. Addressing the growing maize accessions, establishing pan-genes—encompassing gene models or alleles present in a species—becomes crucial. To tackle this, we developed a pan-genomic approach employing representative models derived from a comparative analysis of 38K gene family trees across 26 maize genomes via the Ensembl Compara pipeline. We identified 106K pan-genes, with 20% core, 66% softcore, and 14% shell genes. Additionally, we classified pan-genes based on taxonomic age.Core genes exhibited higher multi-exonic features, on average older in taxonomic age and mRNA evidence than softcore and shell genes. We propagated these pan-gene representatives to 26 maize NAM genomes using Liftoff and improved gene structures using full length transcriptome evidence via PASA. This approach increased average protein coding gene counts to ~60K across maize accessions, increasing sensitivity, enhancing annotations, detecting novel isoforms, and variants. The protein coding set had 33% core, 54% softcore & 13% shell genes. Utilizing the Gramene gene tree curation tool, we visually identified inconsistent gene models, flagging 272 genes for manual curation out of 1167 initially evaluated for B73 v5 models. We further open this curation tool for the community to explore flag genes that are problematic.

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P169 @JFernandezCane1

The establishment and progression of microbial communities associated with the developing seedling roots of maize and sorghum

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All plant seeds carry ectopic and/or internal microbes that can become a major component of the developing plant's microbiome. Very little is known about the biology of the early seedling participants in microbiome communities in any organism or organ, including the root tissues of grasses. We used a time course of shotgun RNA and DNA sequence analysis of root samples taken from maize seedlings transplanted into a field on the University of Georgia campus to identify and quantify the changes ongoing during early stages of seedling development. These stages included replicates for 4 data points in the first 24 hours after these 14-day-old seedlings were transplanted into the field. Three different genotypes of sorghum were studied in parallel, to look for host genotype effects, and another set of identical experiments was performed at the same time with maize inbred B73. A huge diversity of microbes was detected, from all kingdoms of life, but a much lower diversity than seen in the surrounding rhizosphere or adjacent root-free soil. We will present analyses indicating the temporal, successional, functional, and host-species-specific processes that occur in this dynamic, complex environment.

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P170

The maize rhizoplane microbial community

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All plant organs and tissues function in the presence of microbes, some with beneficial and some with pathogenic effects. Most plant-associated microbes, however, are not known to make any contribution to plant biology, at least partly because this question has been so little investigated. The least understood microbial component of plant root environments is the root surface, otherwise known as the rhizoplane. We have used "plating" of the washed root from maize seedlings to isolate bacteria that are associated with the rhizoplane but can be displaced from it by contact with agar medium. As a preliminary experiment, we purified ten independent bacterial colonies from an agar-plate imprint of a washed seedling root of maize line B73. These microbes then had their genomes sequenced by nanopore long-reach technology. Annotation of the ten fully sequenced genomes indicated that they ranged in sizes from 4,109,985 bp to 7,772,202 bp, and that a few were bacteria that we have commonly found associated with maize rhizosphere, like *Pseudomonas glycinae*. However, some represented genera that were not commonly found in the rhizosphere or endosphere, and even represented new species of well-studied genera like *Duganella*, *Flavobacterium*, *Janthinobacterium*, *Luteibacter*, *Pantoea*, and *Sphingomonas*. The majority of these rhizoplane microbes were enriched in chemotactic and/or flagellar genes compared to their closest relatives, suggestive of extensive mobility in the rhizoplane environment. Ongoing studies include analysis of the interactions of these microbes with the root and with other microbes in the soil environment.

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P171

Transcriptomic responses in maize and green foxtail to Poaceae-adapted and generalist lepidopteran herbivores

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Herbivorous insects in the order Lepidoptera cause massive losses to agricultural yields worldwide. One such species, the fall armyworm (Spodoptera frugiperda) causes the greatest damage to maize and exhibits host plant preference for Poaceae species. The beet armyworm (Spodoptera exigua) also feeds on monocots but is more commonly a pest on dicot vegetable crops. Insect feeding induces the accumulation of hundreds of metabolites in plants, however the regulation and biosynthetic pathways of most of these metabolites remain unknown. We compared the transcriptional impacts of two armyworm species on maize and the undomesticated grass green foxtail (Setaria viridis). This was done through a transcriptomic analysis comparing plant local responses four hours after herbivory from the monocot-adapted fall armyworm and the generalist beet armyworm. Results show significant transcript-level increases in plant defensive pathways, including jasmonic acid biosynthesis, jasmonic acid signaling, and secondary metabolic pathways such as indole-derived metabolism. Differences in defense responses between the two herbivores were investigated to identify genes with expression specifically affected by the monocot-selective fall armyworm. Comparisons between the two grasses show shared defensive pathways and divergent strategies between the domesticated and undomesticated plant hosts. Coexpression analysis identified genes with highly correlated expression patterns to herbivory-induced genes, implicating them with potential roles in defense response and regulation of secondary metabolism. These results help to further define herbivory defense in both maize and green foxtail for future investigations of plant-insect interactions.

P172

Unraveling the chromatin environment of double-strand breaks leading to crossovers and non-crossovers

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Meiotic recombination is initiated by the formation of double-strand breaks (DSBs), in chromosomal DNA, which are subsequently repaired to result in crossovers (COs) or non-crossovers (NCOs). COs occur when there is a reciprocal exchange of genetic material between homologous chromosomes. NCO formation, which is how the majority of DSBs get repaired, occurs through DNA synthesis, utilizing either the sister chromatid or the homologous chromosome as the template. Chromatin environment is thought to play a role in controlling the distribution patterns of both DSBs and COs. Interestingly, there are indications that the chromatin environment of DSBs that become repaired into COs are distinct from the chromatin environment of DSBs that result as NCOs. We aim to identify the specific chromatin features that facilitate these two types of repair. To do this, we use machine learning (ML) to analyze high-resolution maps of DSBs, CO-intermediates, and COs. Understanding how the chromatin environment facilitates CO formation will help us design methods to control where COs occur in the genome.

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P173

Utilizing hyperspectral field imagery for accurate southern leaf blight severity grading in maize

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Crop disease detection using traditional scouting and visual inspection approaches can be laborious and time-consuming. Timely detection of disease and its severity over large spatial regions is critical for minimizing significant yield losses. Hyperspectral imagery has been demonstrated as a useful tool for a broad assessment of crop health. In this study, we used off-axis hyperspectral imagery from entire fields, coupled with breeder-assigned plot-wise severity scores from a single growing season, to develop a machine-learning framework to detect and grade the severity of southern leaf blight infection in corn. We formulated a process that (i) enhances the predictability of assigned severity scores and (ii) assesses the interpretability of specific spectral wavelengths by surveying their connections to well-known biochemical processes. We implemented feature reduction methods, e.g., Linear Discriminant Analysis (LDA), L1-Regularization, and Sequential Feature Selector, with a focus on identifying the most discriminative wavelengths. These selected wavelengths were then evaluated using linear and non-linear regression-based models and quantified their effectiveness in identifying and grading disease in corn. The results showed that transforming the feature space with LDA achieves an R2 value of 0.72, however, accepting a reduction in performance (R2 of 0.57) enables the selection of wavelengths through L1-Regularization. Spectral content in wavelengths ranging from 500-550 nm and 675-725 nm have been identified as descriptive and correlate with the production of compounds, such as carotenoids and chlorophyll, highlighting the underlying biochemical mechanisms. Our results show that hyperspectral imagery offers the unique advantage of scrutinizing the intensities of specific wavelengths, allowing us to capture variations in disease grades and their progression. Furthermore, the development of hyperspectral field imagery and machine learning models hold the potential for broader applications in monitoring and mitigating stressors in various crops, thereby advancing food security and promoting sustainability in agriculture.

P174 @Juli Jing

An F-box protein ACOZ1 functions in crossover formation by ensuring proper chromosome compaction during maize meiosis

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Meiosis is an essential reproductive process to create new genetic variation. During early meiosis, higher order chromosome organization creates a platform for meiotic processes to ensure the accuracy of recombination and chromosome segregation. However, little is known about the regulatory mechanisms underlying dynamic chromosome organization in plant meiosis. Here, we describe *abnormal chromosome organization in zygotene1* (ACOZ1), which encodes a canonical F-box protein in maize. In *acoz1* mutant meiocytes, chromosomes maintain a leptotene-like state and never compact to a zygotene-like configuration. Telomere bouquet formation and homologous pairing are also distorted, installation of synaptonemal complex ZYP1 protein is slightly defective. Loading of early recombination proteins RAD51 and DMC1 is unaffected, indicating that ACOZ1 is not required for double strand break formation or repair. However, crossover formation is severely disturbed. The ACOZ1 protein localizes on the boundary of chromatin, rather than directly to chromosomes. Furthermore, we identified that ACOZ1 interacts with SKP1 through its C-terminus, revealing that it acts as a subunit of the SCF E3 ubiquitin/SUMO ligase complex. Overall, our results suggest that ACOZ1 functions independently from the core meiotic recombination pathway to influence crossover formation by controlling chromosome compaction during maize meiosis.

Gene / Gene Models described: *Zm00001d039352*; Zm00001d039352
Funding acknowledgement: National Science Foundation (NSF), National Natural Science Foundation of China

P175

B chromosome mediated effects on A chromosomes

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In the process of introgressing B chromosomes into the sequenced version of B73, once the B copy number was increased, we observed nine different trisomies and one haploid of A chromosomes among 69 seedlings analyzed via root tip squashes. A similar introgression of B chromosomes into W22 did not produce such an effect. The B73 results were somewhat reminiscent of the High Loss (HL) line described by Rhoades and Dempsey in which knobbed chromosomes break at the second pollen mitosis in the presence of B chromosomes together with the production of trisomies and triploids, although the B73 results did not show a high level of chromosome breakage. A study of the High Loss effects was initiated for comparison. When the HL line with multiple B chromosomes was crossed to an *a1* tester, a high rate of breakage of chromosome arm 3L was observed given a large knob there. Also present were many defective kernels. A karyotypic analysis of kernels from this cross revealed triploids, monosomies, trisomies, fragments, and rearranged chromosomes. An examination of the pollen in the HL+B's line revealed chromatin bridges between the two sperm and many grains with only a single sperm. Crosses of the HL+B line to tetraploid females revealed that the single sperm were diploid and functional in fertilization of both the egg and the central cell as evidenced by a higher frequency of normal kernels and karyotype analysis. An experiment with two-day pollinations suggested that the diploid sperm could be present in normal kernels due to heterofertilization. Progeny testing via self-pollination of the kernels of an ear from the *a1* x HL+B cross found that all of the kernels that uncovered the *a1*allele in the endosperm had received the *A1* allele in the embryo indicating that the sperm with the broken chromatid fertilized the central cell. Among the larger class of colored endosperm kernels from this cross, one might expect an equal frequency of semisterile *a1* individuals that received the broken 3L arm in the egg. However, they were present at only ~8% of the reciprocal class. Two selfed individuals had ears characteristic of triploids, which was confirmed by variable numbers of chromosomes between 20 and 30 among the surviving kernels. Both kernel classes of colored and colorless endosperms from this cross produced some spindly plants that were sterile and are of unknown composition. Collectively, the results indicate that the nondisjunction property of the B chromosome at the second pollen mitosis can spill over to the A chromosomes depending on the background with W22, B73, and High Loss behaving differently. While the effect on knobs in the HL line has been known for some time, our results indicate that the centromeres of one or all A chromosomes might remain adhered at the second pollen mitosis to product the high rate of diploid sperm or A chromosomal trisomies.

Funding acknowledgement: National Science Foundation (NSF)

P176

Elucidating the relationship between DNA replication timing within S-phase and the spatial organization of chromatin within maize nuclei

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DNA replication is a temporally regulated process across the S phase of the cell cycle. The timing of replication within the S phase for a specific genomic region is measurable and is referred to as Replication Timing (RT). Aspects of RT are relatively well characterized in animals, but considerably less so in plants. To fill this gap, our group has developed robust technologies to define and characterize the genomic and spatial organization of DNA replication in maize (B73 and NC350) and its close relative sorghum. We discovered that maize euchromatin exists in two distinct "compartments" that are distinguished by their replication timing (early versus middle S) and chromatin status as determined by DAPI concentration. This model is being tested to determine if the two euchromatin compartments are general features of the maize genome throughout the cell cycle. Two orthogonal assays are being used, one using chromosome painting oligos designed from RT classification and another using proximity ligation where intra-compartment contacts between same RT class sequences are expected to be enriched. Oligo FISH painting with RT-specific probes along a representative chromosome arm (5, short) revealed that early S replicating regions and middle S replicating regions occupy largely non-overlapping nuclear regions despite their extensively interspersed arrangement along the linear genome assembly. The early S FISH paint signals were in DAPI weak areas whereas the middle S FISH paint signals were in DAPI strong areas. These findings provide cytological evidence for our published "mini-domain chromatin fiber RT model", now also seen outside of S phase, in G1-stage nuclei.

Funding acknowledgement: National Science Foundation (NSF)

P177

Global modulation of gene expression in aneuploidy combinations in maize

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Genomic imbalance refers to the more severe phenotypic consequences of changing part of a chromosome compared to changing the whole genome set. Previous genome imbalance studies in maize have identified gene expression modulation in aneuploids of single chromosome arms in both haploids and diploids. The modulation of gene expression in more complex aneuploids, e.g. monosomy of one arm and trisomy of another, has not been systematically studied in any organism. In this study, hyperploid heterozygotes of B-A translocations involving two different chromosome arms were crossed together. Then, by crossing these combination lines to a normal female stock, nine genotypes can be produced for each. These will have one, two and three copies of one arm with independent one, two and three copies of the other arm in each of the three genotypes for the opposite arm. For the kernel phenotype, the combination of trisomy + trisomy is easily identifiable phenotypically due to the enhanced smaller kernel phenotype, resulting from chromosome deficiency in the central cell affecting endosperm development. By examining RNA modulation, we discovered that cis genes on varied chromosome arms are generally more dosage-compensated in aneuploid combinations than in single aneuploidy even though some showed a clear dosage effect. For genes on the unvaried chromosome (trans), there is greater modulation in most of the aneuploidy combinations, leading to both reductions and increases in gene expression. The modulation in trans is more pronounced in double monosomy than in double trisomy, aligning with the plant phenotype, suggesting that a greater chromosome dosage change could induce more significant alterations in phenotype and gene expression. Ongoing work involves measuring transcriptome size in aneuploidy combinations that will help us understand whether the more dramatic stoichiometric changes of genes involved in protein complexes in the aneuploid combination would upset global mRNA transcription.

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P178

Stability of chromosome segregation by synthetic maize centromeres

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Synthetic chromosomes enable the engineering of multiple genetic pathways for large-scale genome manipulation. To ensure the faithful transmission of synthetic chromosomes, the synthesis of fully functional centromeres is indispensable. In plants, centromeres are not solely defined by DNA sequences but by a histone variant—centromeric histone H3 (CENH3). CENH3 plays a vital role in specifying centromere positions and loading of centromere machinery to direct chromosome segregation. In our previous work, we showed that an Array Binding Sites locus (ABS) containing LexO motifs inserted into maize chromosome 4 long arm (4L) can recruit LexA protein fused with CENH3 (LexA-CENH3). LexA-CENH3 replaces the canonical H3 and recruits native CENH3 to form centromeres. Centromeres activation at ABS loci transformed chromosome 4 into a dicentric chromosome and initiated Breakage Fusion Bridge (BFB) cycles. At low frequency, 4Ls were released and rescued by ABS centromeres (Neo4Ls) at the same time, demonstrating the functionality of synthetic centromeres. So far, we have screened fifteen Neo4Ls and transmitted them at least two generations without LexA-CENH3. The transmission rates of Neo4Ls can improve over time, possibly correlated with centromere expansion. Nevertheless, Neo4Ls still exhibit instability during plant growth, gametogenesis, or fertilization due to altered genome dosage, centromere-mediated chromosome erasures, or meiotic errors of single-copy chromosomes lacking pairing partners.

Funding acknowledgement: National Science Foundation (NSF)

P179

The long journey of Barbara McClintock's slides defining the NOR

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In the spring semester of 1974 at Indiana University, JB took Marcus Rhoades' Cytogenetics course, which was the last time he taught it. During the course, Rhoades and his assistant Ellen Dempsey used numerous prepared microscope slides to illustrate various chromosomal variations. When Ms. Dempsey retired from IU, she took these slides with her to New York to live in Cornwall on the Hudson. In her holiday letter of 2011, she asked whether anyone wanted the demonstration slides and JB requested them, which arrived in 2012 in Columbia, Missouri. In the summer of 2023, LBK alerted JB that Barbara McClintock might have contributed to this collection. While at the University of Missouri, McClintock had offered slides to Rhoades in a letter dated January 30, 1941 after he had begun his faculty position at Columbia University in 1940 and would initiate a course in Cytogenetics. Later Rhoades moved to the University of Illinois and then to Indiana University. Upon searching through the many slides and their narratives, some were found to be attributed to McClintock. Patrice Albert took pictures of the cells on these slides, and we can match at least one cell to a sketch and photograph in McClintock's 1934 paper describing the nucleolar organizing region (called nucleolar organizing body in the paper) (McClintock, 1934, The relation of a particular chromosomal element to the development of the nucleoli in *Zea mays*. *Zeitschrift fur Zellforschung und mikroskopische Anatomie*). The match to images in this 1934 publication is definitive proof that this slide was used in the definition of the NOR. The affiliation of McClintock for this paper is listed as Caltech. The slide would have been prepared at Caltech by June 1933 when McClintock left and potentially taken with her to Germany from where she submitted the paper and then to Cornell where she returned before she assumed her faculty position at the University of Missouri in 1936. McClintock taught a course in Cytogenetics at Missouri from February to May of 1941. Beginning in the summer of 1941, she took a leave of absence to be a visiting scientist at Cold Spring Harbor Laboratory and a visiting professor at Columbia University in New York City. Thus, at least, the slide journeyed from Pasadena, California potentially to Germany to Ithaca, New York to Columbia, Missouri to New York City to Champaign-Urbana, Illinois to Bloomington, Indiana to Cornwall on the Hudson, New York and finally back to Columbia, Missouri. The other slides attributed to McClintock by Rhoades also focus on the NOR, but the timing of their preparation is unknown, but probably before 1941. The quality of five of the six slides is sufficiently retained to obtain photographs as illustrated together with the narratives of each used by Rhoades and Dempsey. The Caltech slide illustrates the divisibility of the NOR by a translocation with different sizes of nucleoli. The other slides include microspore quartets showing alternate disjunction with and without recombination in the interstitial region as well as adjacent1 disjunction of translocations involving chromosome 6, the site of the NOR.

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P180

Unveiling the molecular mechanism of the B chromosome nondisjunction—a component of its drive mechanism

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In addition to the A chromosomes, maize has a nonvital B chromosome that has been useful in genetic analyses in the history of our discipline. Despite being dispensable, it is maintained in populations by a drive mechanism. One of the components of the B chromosome drive is that its centromere frequently undergoes nondisjunction (probably due to the delayed DNA replication) 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, at least two trans factors are required for the unequal allocation of the B chromosome. Previous genetic analyses showed that an F-box domain containing gene Zm00044a000666 ("666") on the tip of the long arm of the B chromosome is required for nondisjunction. The predicted function of this gene is to bind substrates for ubiquitin-mediated proteolysis. To consolidate the genetic evidence, a complementation experiment was conducted by transforming 666-HA driven by its native promoter and then crossed with a B chromosome harboring a 666 deletion (previously recovered by CRIPSR-Cas9 mutagenesis). The resulting F1 plants were further crossed with the *r1-r* tester and the results showed that the presence of the transgene could complement the edited B chromosome for nondisjunction to a level of the normal B chromosome. To further identify proteins that might interact with 666, we collected mature pollen from three genotypes, including plants containing only the 666 transgene, those with only the 666 deletion, and those with both the transgene and the deletion. Then we used HA antibody to perform Co-Immunoprecipitation followed by mass spectrometry. We are currently analyzing the results of MS to identify proteins that interact with 666.

Gene / Gene Models described: *666*; Zm00044a000666
Funding acknowledgement: National Science Foundation (NSF)

P181 @aimeejschulz

A seed dispersal game: curriculum for teaching plant domestication and adaptation to students of all ages

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We have developed an engaging, flexible curriculum for teaching plant domestication and adaptation to students of all grade levels that meets numerous National Next Generation Science Standards. This activity, using miscellaneous craft supplies and a leaf blower, is both low-cost and easy to implement, and segues well into further lessons on plant science, Indigenous agriculture, plant domestication, plant breeding, and evolution. The activity is also really fun and makes for an engaging, dynamic learning experience that can be used at science workshops and in classrooms. The game starts with students designing, building, and testing seeds developed for dispersal in the wild. Next, students mimic the process of domestication by optimizing their designs to create seeds that are more easily harvestable. With our curriculum, students accomplish the following learning goals:Understand that modern crops were domesticated by Indigenous farmers 10,000 years ago.Learn that limiting plant seed dispersal was a large driver for plant domestication in response to farmers' needs.Visualize that plants use multiple adaptations and methods to accomplish seed dispersal.Identify that traits can be selected upon, both naturally and artificially, and that plant domestication often selects for differing traits than natural selection.We have found that this activity gets students of all ages excited about plants and encourages them to think critically about the roles that domestication has played in our current food system. The entire curriculum (lesson plans, presentation, room setup guide, and supplies list) is available at maizegenetics.net/game.

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

P182

Broader impact through teacher education: Teachers confront the messiness of scientific practice

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How might plant geneticists have a broad impact on science education for diverse populations? Examples of outreach have included development of curricula and workshops for teachers, or support for underrepresented students to conduct laboratory research. Here we describe a different approach. We designed, implemented, and evaluated a "plant genetics unit" to be integrated into formal science teacher education coursework, with the aim of reaching diverse public school students through teachers. The unit focuses on the genetics of disease resistance in Maize and ethylene response in Arabidopsis. Both sets of experiments are conducted in parallel over the course of 3-4 class periods. We piloted the unit in two different teacher education contexts, with a small number of students (n=5; n=6). Students were teachers or student teachers in majority-minority school districts. We collected and analyzed students' lab notebooks and responses to a questionnaire and recordings of classroom discussions. Both implementations presented problems that made the inquiry "messy". In one case, Arabidopsis seedlings didn't grow, and in the other, maize controls did not behave as expected. In the latter, the students discovered segregation of an albino mutation and shifted to understand the inheritance of albinism. Given the logistical problems, we recognize implementing this unit requires greater attention to optimizing conditions, and we are modifying the design to mitigate potential problems. Nevertheless, nine of the eleven students reported that the unit gave them their first real look into the "messiness" of science, and most valued such insight. This work raised questions about whether it is appropriate for adolescents (and/or teachers) to have an "inconclusive" experience with science before having a "positive" experience in which experiments work as expected. We propose that larger-N studies, in which we compare outcomes from conditions of "messy" and "clean" experiments, will provide greater insight into this debate.

P183

CENSA: Create a sustainable food system for nitrogen: Moving synthetic nitrogen from the field to barn

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The flow of nitrogen through the agricultural system is highly inefficient and leaky, resulting in the pollution of our waterways, excessive emission of nitrous oxide from our croplands and manure, methane from livestock, and carbon dioxide from synthetic nitrogen fixation, together accounting for 11% of US greenhouse gas emissions. Agriculture and the food system are projected to become the primary sources of greenhouse gas emissions by mid-century. We argue that by shifting the use of synthetic nitrogen directly from croplands to livestock and human food, we can dramatically decrease this inefficiency. Furthermore, by lessening the nitrogen demand on our croplands, we can promote a circular nitrogen flow in the cropping-soil systems, paving the way for a transition that maintains high productivity while lessening environmental harm.

Funding acknowledgement: United States Department of Agriculture (USDA)

P184

Characterizing and distributing maize diversity: The NCRPIS maize collection

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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 75% of the collection (15,358 accessions) is available for distribution upon request and 82% of the collection is backed-up at the National Laboratory for Genetic Resources Preservation. Seed viability of distributable seed lot 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. Nurseries provided by partners and contractors in the US and Mexico are used 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 367,327 trait observations on 17,428 accessions, and 43,452 ear, kernel, and cob images on 16,804 accessions. Germplasm requests can be made through the NPGS GRIN-Global public website. In FY2023, 14,738 packets of maize were distributed by NCRPIS to requestors across the country and around the world.

Funding acknowledgement: United States Department of Agriculture (USDA)

P185

Evolution of genome editing regulations: Comparative analysis in model countries

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Genome editing technologies have surged forward in biotechnology, revolutionizing scientific possibilities distinct from conventional genetic modification methods. This research conducts a comparative analysis of regulatory frameworks governing genome editing in Model Countries—England, Brazil, Canada, Australia, and Japan—uncovering distinct approaches and developmental trajectories.Utilizing an exhaustive review of legislative mandates, policy guidelines, and scholarly literature, this study highlights regulatory nuances among these five nations. It examines legal structures, ethical considerations, and developmental timelines, emphasizing the growth and diversity in genome editing regulations.Initial findings reveal a spectrum of regulatory approaches, ranging from stringent frameworks necessitating comprehensive risk assessment to adaptable policies fostering innovation and safe utilization. Understanding these variations is vital for policymakers, scientists, and stakeholders, aiding informed decision-making and potentially promoting alignment in global regulatory standards.This comparative analysis illuminates the dynamic evolution of genome editing regulations, offering insights into how nations navigate the ethical, legal, and societal dimensions of this burgeoning technology.

P186

From the Inari gene editing toolbox: CRISPR/Cas-mediated enhancer insertion in maize

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Because of climate change, we have no other option but to feed our growing population with less land and fewer inputs. At Inari, we combine predictive design and an ever-deeper understanding of the plants' complex inner workings with multiplex gene editing to drive improvements in yield and resource use efficiency. Our gene editing toolbox consists of technologies that enable us to turn specific genes off/on, tune their expression up/down and facilitate sequence replacement. Here, we demonstrate the use of the CRISPR/Cas system beyond traditional gene disruption, to upregulate gene activity. We identified a small element native to maize, that upon integration in target gene promoters enhances gene expression. Insertion into the promoter is achieved during non-homologous end joining-mediated repair of a CRISPR/Cas-induced double stranded break. Across all 11 target genes, insertion efficiencies averaged 29% (range 6%-61%). In all examined cases, enhancer insertions identified in T0 plants were inherited by the T1 progeny. Expression analysis in resulting T1 populations demonstrated increased gene expression in leaves for all targets except one and in primary roots for all targets; even genes with high basal expression could be further upregulated. As a proof-of-concept, we applied our CRISPR/Cas-mediated enhancer insertion technology to upregulate genes involved in nitrogen use efficiency (NUE) and performed small-scale phenotyping experiments. When grown in limited nitrogen, maize plants with increased expression of ALANINE AMINOTRANSFERASE, a cereal NUE gene, suggested higher tolerance to low nitrogen compared to siblings without the enhancer allele. Furthermore, we showed that the insertion of the enhancer element can be combined with other types of edits such as small insertions or deletions in other genes. To conclude, the Inari gene editing toolbox contains different technologies that can be combined to enable advanced engineering of complex traits, and to correct deleterious alleles to improve sustainable maize production.

Gene / Gene Models described: *Gln1-3, NLP5, ENOD93, NAC60, AlaATb, Dof1, GOGAT1, AspAT, NAC49, AlaATa, GS2*; Zm00001d017958, Zm00001d015201, Zm00001d045390, Zm00001d013003, Zm00001d014258, Zm00001d031278, Zm00001d043845, Zm00001d043382, Zm00001d034601, Zm00001d030557, Zm00001d026501
Funding acknowledgement: VLAIO (Flanders Innovation & Entrepreneurship)

P187

Leaf angle and curricular innovation: a CURE for research and undergraduate education

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Undergraduate research is arguably the single most important experience for inspiring students to pursue graduate school and STEM careers. Yet, most students never have the opportunity to engage in research simply because of time and limited institutional resources. Course Based Undergraduate Research Experiences (CUREs) address these issues since students and institutions are already committed to classes. CUREs are not new to undergraduate curricula but they often occur as a singular opportunity as opposed to a curricular foundation, and therefore lack the impact of a prolonged research experience. To address this issue, we have developed a four-year CURE program that consists of eleven courses spanning four STEM majors. Students experience a breadth of research systems, questions, approaches, and analyses at every developmental level and the scale of the program ensures that a significant portion of every student's education is research based. Here we present an overview of our CURE program and then illustrate how undergraduate researchers are helping to identify transcription factors that control leaf angle in maize. Leaf angle is an important trait that has historically been manipulated by breeders to increase planting density and therefore yield. Since the auricle contributes significantly to leaf angle, undergraduates helped perform a yeast one hybrid screen using the promoters of NARROWSHEATH1, LIGULELESS1, LIGULESS3, and ROUGHSHEATH1; all genes that are expressed in the pre-ligular band during leaf development. Surprisingly, all of these genes were bound by some combination of only four transcription factors in the Lateral Organ Boundary (LOB) family, including RAMOSA2. The small number of transcription factors identified as well as the overlap suggests that these four genes are in the same regulatory network and function early in auricle development. This finding illustrates how undergraduate education, CUREs, and discovery can be joined to the benefit of all parties.

Gene / Gene Models described: *NS1, RS1, LG1, LG3, RA2*; GRMZM2G069028, Zm00001eb299420, Zm00001eb067740, GRMZM2G087741, Zm00001eb123060

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P188

MARVEL: An inclusive student persistence framework to close equity gaps in STEM workforce development

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Student retention in STEM majors is critical to meet an increased demand for STEM graduates within the workforce. Student retention challenges, particularly for underrepresented groups, often include barriers impacting student self-efficacy, persistence, motivation, and STEM identity. To address these issues and promote student persistence in STEM, the MARVEL (Microbiology Achievement through Research and Valuable and Experiential Learning) program was developed as an intervention to address these barriers impacting student persistence by fostering community, building confidence, and providing early experiential learning opportunities. Targeted interventions include embedding student participation in research symposia, experiential learning, and professional development-oriented workshops into the curriculum of a first semester majors' course, as well as application and reflection of learning outcomes to students' specific career preparation plans. Through participation in these activities, student confidence and sense of belonging increased as students saw themselves as part of a vibrant scientific community and able to network with other students, faculty, and staff with similar interests. Through the guided reflections, students also were able to connect the value of participating in these activities to their own career preparation goals. With continued participation throughout their academic programs, students will continue to build their confidence by developing a portfolio of professional development skills while networking and building connections to enhance their chances for future success in the workforce. The MARVEL program provides a model for other STEM majors and programs to promote inclusive access to professional development, experiential learning, and networking activities while fostering community and providing workforce development opportunities for students within their programs.

P189 @cornqueenb

The *Maize-10-Maze* project, an educational public chromosome map garden with mutants of maize at FSU and online.

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The *Maize-10-Maze* is a public outreach event representing the ten chromosomes of maize in a live field museum of select fun and famous mutants of maize. Positioned within a cornfield with a walking path and information placards, families segregating for dominant or recessive mutants are planted in chromological order in which each row represents a single chromosome with ~7-10 mutant families. Our most recent *Maize-10-Maze* was held in Tallahassee FL in the summer of 2023, and replicated at other institutions (The Danforth Center in St. Louis, MO; NC State University in Raleigh, NC). These chromosome gardens provide opportunities for training and educating the scientists and students who produce and host them. Here we report on the June 2023 *Maize-10-Maze* event, a cornerstone outreach event of our maize DNA replication project (NSF IOS 2025811). The selected mutants showcased visually striking phenotypes/traits or those with agronomic or scientific importance, such as *Knotted1 (Kn1), lazy plant1 (la1), brittle endosperm1 (bt1)*, or *teosinte branched1 (tb1)*. The site offered tours, either self-guided or hosted by FAMU/FACE students, and exhibited mutants with detailed placards (FSU 2023 placards & pheno-pics) containing links to maize genetics. This year, the Young Scholars Program (YSP) high school students added fun-fact centromeres to each chromosome. In collaboration with Florida A&M University's FACE Summer Program, YSP, and videographer Jonathan Doster, we created engaging digital content about maize mutants and plant genetics. Public access continues to be facilitated through various outlets, including a dedicated website, www.crazylazycorn.org, social media channels such as Instagram (@cornqueenb) and TikTok (@scienceforyall), online placards document, OMEKA (crazycornmutants.omeka.net/), a virtual mutant museum, curated by students for a worldwide audience. This project serves as a captivating opportunity for public engagement with modern maize research incorporating molecular genetics, genomics, and plant biology.

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P190

A MAGIC Population to study functional diversity in mexican native maize

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Native Maize Varieties (NMV) are adapted to their specific agro-environmental niche and local management practices. In México, NMVs are grown in a highly diverse set of environments, from hot and humid, to cold and arid, and, as such, represent an important resource to study the genetic basis of adaptation of crops to extreme and contrasting environments. Historically, the study of the genetic architecture of quantitative traits in NMVs has been based on the use of biparental mapping populations or diversity panels. In this work, we present a Multi-parent advanced generation intercross (MAGIC) population generated with the genomic contributions of 8 Mexican NMV donors (MEXIMAGIC population) representative of the agro-climatic and environmental conditions where maize is grown in Mexico. A pilot set of 164 families of the MEXIMAGIC population were test-crossed with NC358 and the F1 hybrids were evaluated in a field experiment. 3 representative ears per row were scanned and the ear length, width, RGB spectrum (pigmentation) and perimeter principal component values were obtained from the images. Ear and Cob phenotypes were used as phenotypic input for Genome-wide association and Quantitative Trait Loci (QTL) analyses. 5 QTL were identified in both analyses (alpha = 0.1): 2 for cob pigmentation, 1 for cob width, and 1 for ear width. In addition to the mapping results, we discuss the design, development, genotyping, and future expansion of the population. The identification of QTLs and their allelic effect works as a proof of concept for the potential of the MEXIMAGIC to dissect the genetic architecture of quantitative and agronomical relevant traits in NMVs.

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

P191

A MYB transcription factor underlying plant height in sorghum and maize reveals functional connections across natural alleles, mutant, and edited alleles

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Manipulating plant height is essential for crop improvement. Plant height was generally reduced through breeding in wheat, rice, and sorghum to resist lodging and increase grain yield, but kept high for bioenergy crops. Here, we positionally cloned a plant height quantitative trait locus (QTL) *qHT7.1* as a MYB transcription factor controlling internode elongation, cell proliferation, and cell morphology in sorghum. A 740 bp transposable element insertion at the intronic region caused a partial mis-spliced transcript isoform with an additional exon, which contained a premature stop codon and led to a truncated amino acid sequence with an altered C terminus. Gene expression analysis revealed a dynamic pattern of *qHT7.1* across development and along the internode position. 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 in two maize inbred lines showed significant plant height reduction. The overall discovery enriched our understanding of plant height regulation in sorghum and maize, and enhanced our toolbox for fine-tuning plant height for crop improvement.

Gene / Gene Models described: *br1 (maize); qHT7.1 (sorghum)*; Zm00001d032194; Sobic.007G137101
Funding acknowledgement: United States Department of Agriculture (USDA), Iowa State University Plant Sciences Institute, Iowa State University Crop Bioengineering Center, Iowa State University Raymond F. Baker Center for Plant Breeding

P192

A Rootless1 knockdown allele affects maize nodal root development, changing root system architecture and function.

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Nodal roots are the dominant part of the maize root system and are important for nutrient acquisition and physical support. Changes in nodal rooting patterns could help improve root system function, for example, the "Steep, Cheap, and Deep" paradigm posits that high occupancy nodes at, or just above, the soil line, but no higher, have benefits to efficient nitrogen and water capture. Rootless1, a classic maize mutant first described in 1930 and later mapped to Chromosome 3S, produces very few nodal roots aboveground. We previously identified a large indel in the promoter of ZmRt1 that reduces expression and which we hypothesize is responsible for the original rootless1 phenotype. However, an Ac/Ds knockdown allele of ZmRt1 named Rt1-2, was observed to induce supernumerary nodal roots close to the soil line before a precipitous decline at higher nodes which we hypothesize to result in changes in the root system, improving nutrient acquisition abilities. A comprehensive multi-year analysis of altered nodal root development and changes to root system architecture was conducted. A nitrogen contrast field experiment was performed in 2022 and 2023 comparing the Zmrt1-2 allele to the ZmRt1 wild type allele in high and low nitrogen conditions. Root crowns and 1m deep soil cores were taken to determine root distributions and root length density across the depth profile. Plants containing the Rt1-2 allele demonstrated changes in root length density, suggesting changes in nodal rooting affect root system exploration in the soil. Aboveground measures including biomass, total plant nitrogen, and yield were measured. Plants containing the Rt1-2 allele demonstrated higher yield in both high and low nitrogen conditions than the ZmRt1 wildtype allele, suggesting a positive influence on root resource capture efficiency.

Funding acknowledgement: National Science Foundation (NSF), Valent Biosciences

P193 @Natekorth

A case for GERM: calculating Genotype by Environment by Rhizosphere Microbiome interactions to improve crop yield and resilience.

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Genotype by Environment interactions in maize have been extensively studied to understand genetic drivers of environmental adaptation, still many mechanisms of GxE remain elusive. The root associated microbiome (rhizosphere microbiome) benefits the host plant by increasing nutrient availability, growth via plant hormones, and resistance to pathogens. These benefits can be thought of as an expansion of the genomic and metabolic capabilities of the host plant. The plant microbiome is determined by host genetic and environmental factors. Many studies treat the microbiome as a phenotype of plants impacted by plant genotype and the environment. While this is useful for elucidating genetic and environmental factors that shape rhizobial communities, we argue the rhizosphere microbiome should be treated as a separate variable in determinations and predictions of plant phenotypes such as yield and resilience to climate stress. We propose a pilot scale experiment to address the hypothesis that the three-way interaction between maize genotype, environment and the rhizosphere microbiome significantly impacts maize stress tolerance while uncovering maize-driven microbial mechanisms of environmental adaptation.

P194

A cat-and-mouse game for the stay-green phenotype in maize hybrids

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The stay-green trait enables an extended photosynthetic period after anthesis which lengthens the plant's grain filling period contributing to an increase in yield. This study was conducted to determine which hybrids stayed green for longer periods of time compared to other hybrids in the same location. Identifying hybrids that stayed green longer can give breeders valuable insight about which genotypes they should use in introgression efforts. At the end of physical maturity, optimum differences in canopy senescence can be observed. Once a week, plants were scored on a scale from 1-9 (9 being a full green canopy and 1 being completely senesced) and a senescence percentage (in increments of 5%) was recorded at the West Madison Agricultural Research Station in Wisconsin on 2 replications of 250 genotypes. In the 2023 growing season, disease was at a minimum and conditions were dry, equating to the ideal environment to observe senescence patterns. The selection of the top hybrids that stayed green the longest was based on the mean ratings from the last date measurements were collected. From highest to smallest ranking, the top hybrids were: PHK93 X PHP02, LH123HT X PHP02, PHK93 X PHP02, PHK76 X PHP02, and PHG35 X PHP02. All plants were completely senesced by October 27, 2023.

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P195

A comparative approach for selecting orthologous candidate genes underlying signal in elemental accumulation genome-wide association studies across multiple species

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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 verified 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.

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P196 @cornontherocks

A new ethanol extraction method in maize using lab-scale, dry-grind technology

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Understanding maize genetic diversity has become an exciting research avenue that will result in new ways to add value to maize as a crop. As biochemical and genomic tools become more high-throughput and cheaper, research can answer questions regarding maize diversity. One such question is how diversity can be leveraged to make better food and beverage products that rely on maize as the primary ingredient. To answer this question, we are exploring metabolite diversity in heirloom varieties and in corn whiskey produced from these heirlooms, and using QTL mapping approaches to identify genetic loci controlling production of these metabolites. We developed a platform to efficiently produce new-make whiskey from small batches of heirlooms in order to quantify metabolites that contribute to flavor. Typically, current lab-scale methods require larger quantities of seed (on the order of 500 grams to 1 kilogram) to produce whiskey and are not optimized for low yielding heirlooms or non-elite populations. Our new method is a scaled-down version of the industrial distilling process that minimizes seed input (100g, or approximately 1-2 heirloom ears) while producing sufficient distillate (about 14 mL). This method mimics industry production in the US and is effective at producing ethanol at a genetics/breeding research scale. We have validated the method using ethanol and sugar quantification assays to effectively measure the success of anaerobic fermentation. This platform allows for further study of metabolic diversity in heirlooms and serves as a methodology for quantifying kernel carbohydrates for ethanol production in maize.

Funding acknowledgement: University of Missouri

P197 @freeaSarahgene

A tale of two functions: Exploring Asparagine's impact on human health and the environment

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Free asparagine (Asn) is a critical nitrogen (N) transport and storage compound that can account for a large proportion of the free amino acids in maize kernels. While Asn plays a critical role in plant growth and development, excess N in the grain contributes to environmental problems as excess N in livestock waste can pollute waterways. In addition, when maize is prepared for human consumption, Asn is converted into the probable dietary carcinogen, acrylamide, during the Maillard reaction. For these reasons, we wish to understand the genetic architecture of free Asn asparagine accumulation in maize seeds. Ultimately, we seek to reduce the environmental impact of excess grain nitrogen and improve human health by reducing the precursor to acrylamide. There is genetic variation for both free Asn and protein content in diverse maize. This research aims to identify the genetic determinants of free Asn accumulation in maize seeds using genome-wide association studies (GWAS) and transcriptome-wide association studies (TWAS). Preliminary GWAS studies identified 421 gene regions related to Asn accumulation, while TWAS identified at least 95 genes. Interestingly, there were no overlapping association signals between GWAS and TWAS, suggesting that either different environments yielded variable results or that gene expression is not the only underlying mechanism. Beyond gene identification, we will use RNAi and overexpression of prioritized candidate genes to tease apart Asn metabolism (biosynthesis and degradation) in the plant and the grain. This holistic approach will inform strategies for manipulating Asn levels, balancing environmental sustainability and human health.

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P198

AI-driven image-based high-throughput phenotyping and genetic analysis of plant architecture in maize

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Breeding maize for increased grain yield has resulted in substantial changes in plant architecture-related traits, including more upright leaves that decrease mutual shading and provide increased tolerance to higher plant densities, altered plant height via altered numbers of nodes or lengths of internodes, higher harvest index, easier for machine harvest. High-throughput image-based phenotyping of whole plants used in prior studies either sampled destructively or involved greenhouse-grown plants, which may not match field-grown plants' architectures. During 2021-2023, we have applied AI-driven image processing algorithms to extract multidimensional traits from field-grown plants imaged non-destructively using a Phenobot. Recent improvements to the Phenobot include attaching five tiers of stereo cameras to the Phenobot's mast at different heights to ensure they capture the same plants' images, simplifying data processing. Custom-made strobe lights, which provide stronger illumination at a lower cost, were added to each stereo camera. Distance-based triggering of camera shutters was employed during the 2023 data collection, reducing the number of duplicate images as compared to our previous time-based triggering method. The Phenobot was used to collect the leaf angle data from the SAM diversity panel. The SAM panel consists of ~380 genotypes, which previous research established exhibit diverse shoot apical meristem traits [1]. Because the entire above-ground plant is derived from the SAM, we anticipated that this diversity in SAM architecture would be reflected in diverse plant architecture. Our preliminary data support this hypothesis. An analysis of a subset of the leaf angles of a subset of the SAM panel reveals diverse leaf angle patterns across genotypes. These patterns of leaf angles match ground truth data collected from destructively sampled plants. The long-term goal of this study is to understand the genetic control of the canopy-related traits and the effects of these traits on the efficiency of light interception by the canopy.

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

P199

Analysis of B73 seed phenotype changes in response to tropical conditions

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B73, developed at Iowa State University, is a cornerstone in maize research, embodying the Iowa Stiff Stalk Synthetic population and contributing to the success of the Reid yellow dent maize group with its superior grain yield, adaptability, and strong stalks. Serving as a reference genotype, B73's stability and well-characterized genome have played a pivotal role in maize genetic studies, genetic mapping, and genomic research, particularly in molecular breeding and functional genomics, following its comprehensive sequencing under the Maize Genome Sequencing Project. In the absence of a thoroughly sequenced and well-assembled tropical maize inbred line serving as a genomic reference, B73 stands out as the optimal reference, even for tropical genetic backgrounds. We introduced B73 to Brazil through a seed sample generously provided by the Maize Genetics Cooperation Stock Center. Two consecutive cycles of self-pollination were conducted in the summers of 2018-2019 and 2021-2022. Subsequently, seeds from each progeny were assessed for weight, area, diameter, and color, revealing a discernible difference in weight between B73-S1 and B73-S2. The seed size progressively expands across generations, as indicated by Feret's diameter, aligning with the observed pattern of seed areas exhibiting a similar trend. The progenies exhibit a subtle variation in seed color, although it lacks statistical significance. Abortive plants were observed in the field, though the frequency was not quantified. Our findings indicate that certain traits may exhibit variations across generations in response to environmental conditions and contribute to adaptation in tropical regions.

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P200

Analyzing the impact of 40 years of breeding and low nitrogen environments on maize root system architecture using X-ray imaging

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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. This study examined the impact of maize breeding, environmental variation and nitrogen availability on RSA through comparisons of root traits from a panel of era hybrids. Twenty-seven maize hybrids were selected from across four decades (1985-2020) of the Bayer Crop Science breeding program and planted a control fertilizer rate (200 lbs./acre N) and a low rate (50 lbs./acre N) at three different fields in Iowa, Indiana, and Illinois. To explore differences in RSA, in the summers of 2021 & 2022, we shovel-excavated 1300 root crowns at stage R3, averaging 6 samples per era hybrid at each N rate and field site. We generated digital 3D reconstructions using X-ray computed tomography and used a custom computational pipeline to further distinguish their overall contributions and impacts upon individual traits. From these reconstructions, we calculated more than 100 root traits in 3D such as total root length, total volume and number of root tips. Additionally, we measured the local climates, soil nitrogen, bulk density and water table depth throughout each season along with soil texture at each field site to better understand which environmental parameters were correlated with the changes in RSA development. Subsequent analysis shows that breeding, environmental variation and nitrogen availability all account for root crown phenotypic variation and we are working to further distinguish their overall contributions and impacts upon individual traits. 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 in turn can help improve future maize hybrids.

Funding acknowledgement: Foundation for Food & Agriculture Research (FFAR)

P201

BZea: A diverse teosinte introgression population for investigating agronomic traits in maize

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Teosinte, the wild ancestor of maize, harbors a rich reservoir of adaptive traits that have been largely diminished during maize domestication. Recognizing the potential of these unknown alleles, here, we present the development and preliminary characterization of a new teosinte introgression population. The donor lines of this germplasm 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 5-15 derived lines per most of the accessions. 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, entitled "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. This also streamlines the analysis of agronomically relevant traits such as cold tolerance and disease resistance. To characterize this population, we conducted whole genome sequencing of 1400 of these derived lines with an average sequencing depth of 0.8x. We then used B73 as a reference for mapping to apply the hidden markov model (HMM) and identify the introgressed regions. We also performed a field evaluation of the population in the summer of 2023 consisting of 3 replicates of around 1200 derived lines and collected morphological, physiological, and photosynthetic phenotypes. Building on a wide sampling of teosinte diversity, preliminary analysis of the results indicates the potential of teosinte alleles to expand the phenotypic space of important maize agronomic traits.

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P202

Breeding corn for organic production systems

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Organic meat and egg producers must use organic ingredients in feed, creating a great demand for organic corn. We are developing corn varieties to meet the needs of organic corn producers. We test varieties in organically certified fields and select for characteristics that are important to organic corn producers. Breeding targets include agronomic performance, nutritional quality, ability to produce seed in the absence of herbicides and natural resistance to insects and diseases. In addition, we incorporate genetic systems such as Ga1-S that allows exclusion of unwanted pollen and SHGD (spontaneous haploid genome doubling), that confers the ability to produce doubled haploids without chemical treatments that are banned in organic systems. Our breeding program includes a rapid cycle method that uses doubled haploids and genomic selection to greatly reduce the time required to complete a breeding cycle.

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P203

Breeding for color and improved protein content in sweet corn and popcorn

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Zein proteins dominate endosperm protein content of sweet corn and popcorn. However, zeins are deficient of essential amino acids such as lysine and tryptophan. The mutation in opaque-2 (O2) transcription factor that regulate protein formation in maize endosperm reduces the levels of alpha-zeins, thus increasing lysine-bound non zeins through proteome rebalancing. Building on insight gained from our previous Quality Protein Popcorn (QPP) project, this project aims to breed publicly available quality protein sweet corn (QPS) and colored QPP varieties. Modified *o2 mutant* maize, also known as Quality Protein Maize (QPM) were crossed to *sugary-1 (su-1)* and *shrunken-2 (sh2)* sweet corn verities and colored popcorn varieties. Colored QPP introgressions had progressed through selfing to advance to the next generation; backcrossing (BC) to recurrent popcorn parents to retain optimal popcorn background while selecting for vitreous kernel (modified) lines carrying the gamma zein o2 modifier gene. QPP lines are currently advanced to the final BC3 generation which will be followed by several rounds of selfing to generate inbred lines. Then the inbreds will be selected to produce QPP hybrids.For QPS breeding, F2 kernels visibly segregating for both *o2* mutation and sweetcorn phenotype had been advanced through the F5 and had been selected for homozygous o2 mutation and field selected for sweetness and texture. In addition to protein quality, we aim to improve sweet corn micronutrient content and aesthetic appeal by breeding for color diversity. Therefore, sweet corn varieties were crossed to various color donors such as popcorn, dent corn, and flint corn and had been similarly advanced through the F5 generation with several rounds of selection for color, sweetness and texture at 20 DAP. Several standout QPS and colored sweetcorn lines have been identified and will be used in the production of hybrids. In the meantime, F4 and F5 kernels of colored sweet corn and QPS were collected at 20 days after pollination (DAP) and frozen for biochemical testing for sucrose, glucose and fructose, total starch, amino acids, resistant starch, and microbiome characteristics. Thus far, we have completed sucrose, glucose, and total starch analyses for both colored sweet corn and QPS and amino acids analysis for QPS is in progress. We will present summaries of our finds in the poster.

P204

Breeding for root traits in cereal crops

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Optimization of root traits is a promising avenue for mitigating edaphic stresses such as drought, salinity, and suboptimal nutrient availability in cereal crops. Furthermore, crop root systems have the potential to contribute significantly to carbon sequestration to combat climate change . Significant variation for root anatomical and architectural has been found in major crops including maize, rice, and wheat. This variation can be exploited by incorporating root traits into the existing breeding programs. Here, we define a road map on "*How to breed for root traits*?". First, we discuss the major hurdles breeders face when including root traits in their breeding programs, including the difficulty of phenotyping due to the underground nature of roots, high plasticity in root traits. Considering these difficulties, we describe 1) a selection of environment specific root ideotypes that includes both root anatomical and architectural phenotypes. For new trait discovery, the most promising germplasm, such as synthetic mixes, landraces, and wild relatives, can be used depending on the trait of interest. 2) We show the value of functional-structrual root modelling to explore the phenome landscape. We also emphasize a detailed physiological understanding of a trait, including tradeoffs, plasticity, pleiotropy, and phenotypic interactions. 3) We describe high throughput phenotyping platforms such as shovelomics, anatomics, and LEADER (leaf elemental accumulation for deep roots) to phenotype the root traits of interest and integration of these protocols into genomic prediction models. Furthermore, we describe the use of potential proxy traits which can be used estimate complex root functions like rooting depth. As a proof of concept, we discuss successful examples of breeding for root metaxylem vessel area in wheat and root hairs in common beans. We also describe a program we are establishing to select on roots traits in maize.

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P205

CERCA - Circular Economy that Reimagines Corn Agriculture

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Maize is the number one production crop in the US and globally, supporting the global food supply as well as portions of the energy system. Nitrogen fertilizer is the largest energy input for maize, and excess nitrogen results in water pollution and contributes 6% of the entire US GHG (55% of agricultural emissions) through nitrous oxide release. To ensure the sustainability of agricultural systems, improvements to genetics and management practices that would minimize maize's contributions to climate change and water pollution are necessary. A team of scientists from across the US is working together to start solving this problem. Their research aims to create a Circular Economy that Reimagines Corn Agriculture (CERCA) - converting maize to an earlier season annual with reduced environmental impacts through increased uptake and recycling of nitrogen and phosphorus fertilizer. The team is organized in three highly integrated activities: (1) Modeling of plants, farms, environments, and economics to determine the most important combinations of traits and environments likely to benefit from new cropping systems. (2) Trait Discovery through evaluation of related wild species and maize landraces that recycle nutrients and have some cold tolerance. (3) Trait Development through stacking and testing of genetic improvements predicted to have substantial impact. This project is starting with seven native trait concepts focused on greater on-farm nutrient recycling: nitrogen remobilization to roots, reduction of nitrogen and phosphorus in the kernel, biological nitrification inhibition, germination and seedling cold tolerance, and seedling establishment in cool temperatures. CERCA reimagines the cycle of planting and fertilization without changing most of the existing equipment and processes, thus making adoption more likely and efficient.

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P206
CIMMYT Maize Lines (CMLs): A fundamental resource for maize breeders and geneticists

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CIMMYT Maize Lines (CMLs): A Fundamental Resource for Maize Breeders and Geneticists Alejandro Velazquez, Alberto Chassaigne, Cristian Zavala* [1]CIMMYT Int., j.velazquez@cgiar.org, [1]CIMMYT Int. a.chassaigne@cgiar.org, [1]CIMMYT Int. c.zavala@cgiar.org. CIMMYT Maize Lines (CMLs) are a carefully curated collection of 647 elite lines, actively developed by the CIMMYT Global Maize Program since 1991. These lines represent a widely utilized resource of freely available, elite germplasm for both public and private sector maize breeders worldwide. Since 2005, the responsibility for the conservation, regeneration, and distribution of this crucial genetic resource has resided with the CIMMYT Maize Germplasm Bank (BGM). Notably, CMLs constitute the most frequently requested accessions within the BGM collection, requiring more frequent regeneration cycles. Over the past five years, the CIMMYT BGM has forged collaborations with over 100 national institutions, public and private sectors, along with independent producers and universities. These collaborations have addressed all seed requests emanating from the BGM collection, of which more than 50% were for CMLs. Within the framework of the MasAgro-Biodiversity Seed of Discovery (SeeD) Project, we are engaged in a comprehensive evaluation of the genetic diversity encompassing all 27,500 maize, teosinte, and tripsacum spp. accessions housed within the BGM. This endeavor aims to culminate in the development of a comprehensive molecular atlas of maize. Such an atlas holds immense potential to enhance our understanding of maize genetic resources, encompassing landraces alongside advanced and elite breeding lines. Ultimately, this will facilitate the selection, utilization, and further research involving these valuable materials. Genotyping serves as the cornerstone of data unification, critical for its subsequent use in constructing molecular atlases. Concurrent with the BGM accession genotyping evaluations, a more in-depth assessment of CMLs was conducted. This assessment aimed to detect any potential divergence between the original seed received by the BGM in 2005 and subsequent regenerations. Additionally, the objective was to establish a standardized genetic fingerprint for routine quality control analyses. This will ensure the unwavering purity of each CML line following each seed increment or regeneration cycle. The present work details the outcomes of this analysis and its subsequent application in the development of a CML genotyping platform.

P207

Characterizing heirloom maize of the United States

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Hybrid yellow dent maize has stood the test of time on the domestic and international markets, generating billions of dollars for fuel and livestock feed. Open-pollinated maize heirlooms (landraces) are incredibly genetically diverse, both between and within populations, and harbor unique alleles not present in modern cultivars that have experienced intense directional selection. Historically, heirlooms were grown throughout the world for subsistence farming and livestock feed before the advent of modern hybrids. Heirlooms are prized in niche markets, with smallholder farms, chefs, and organic farms interested in their unique and broad culinary applications. Heirlooms from other countries have been intensively studied, with extensive monographs written about landraces from Mexico, South America, Asia, and Europe; however, there is no monograph from the United States. Data that does exist for US heirlooms is largely incomplete and lacks defined population structures based on phenotypic and genetic data. This project will systematically characterize about 1000 heirlooms, representing most of the open-pollinated varieties from the US and Canada held in the North Central Regional Plant Introduction Station. Manual and unmanned aerial vehicle technologies will be used to collect plant architectural traits, and a digital imaging pipeline will measure ear and kernel morphologies, with emphasis on end-use traits. Near-infrared spectroscopy will assess kernel composition traits, including starch, protein, and oil. Pool-seq technology will be used to genotype 500 heirlooms, which represent a large portion of the genetic diversity present in the set. Genetic data will provide insight into the population structure of heirlooms with similar names and morphologies, delineating natural groupings within the set. Subsequent GWAS integrating historical weather, phenotypic, and genotypic data will identify genes involved in adaptation and morphology. Ultimately, this project will produce a publicly available, comprehensive data set describing US heirlooms and the genetics involved in adaptation and quality-related traits.

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P208

Cold tolerance evaluation in maize through seedling establishment strategies

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Maize production continues to grow towards the northern portions of the US Corn Belt. Cold tolerance during germination and seedling establishment poses challenges in those regions that experience cold temperatures in early spring. Optimizing strategies for maize production is crucial for sustainable agriculture. This study aims to improve maize seedling establishment and tolerance as key components of a strategy to extend the growing season to support greater light interception and higher dry matter accumulation.This research focuses on evaluating the cold resistance of seedlings from four biparental mapping populations including 250 double haploids (DH) from a six parent Stiff Stalk MAGIC population, 74 recombinant inbred lines (RILs) from the cross of Gaspe and B73, 130 RILs from P39 by B73 and 181 from B97 by B73. The evaluation also included 63 maize landraces adapted to altitudes above 2500 m. The experiment, designed as a randomized complete block design, comprised single row plots with 20 seeds per plot and two field replicates and it was planted in September at the West Madison Ag Research Station in Madison, Wisconsin to impose cold conditions during seedling growth. The phenotypic evaluation included seedling cold tolerance and regrowth after a cold episode, rating seedling growth in a scale of 1 to 4, where 1 show yellowed and weak plants and 4 green and vigorous. The evaluation also included a drone-based assessment of different parameters such as growth indicators, parallel evaluation of growth stages, with a classification of cold response after two cold episodes (40° F and 30° F). The results of this study are expected to contribute to the optimization of maize production strategies in cold climates to improve seedling establishment, cold germination, and tolerance, thus extending the growing season and promoting higher light interception and dry matter yield in maize.

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P209 @MVianna11

Comparing k-mer-based and SNP-based GWAS approaches to identify structural variants in sweet corn

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Structural variants play an important role in adaptation and evolution. Those polymorphic regions can influence gene expression, epigenetic marks, and, consequently, the phenotype. Genome-wide association studies (GWAS) are commonly used to find candidate genes associated with a trait of interest in polyphonic regions. However, we hypothesize that the SNP-based GWAS may miss important genomic regions associated with structural variation (SV) types when the single nucleotide polymorphism (SNP) is not in linkage disequilibrium with the causal SV variant. An alternative approach is to use short sequences, k-mers, as variables in the GWAS analysis. Here, we compare k-mer-based and SNP–based GWAS approaches to find candidate genes associated with important traits in sweet corn. We used ~9X coverage whole-genome short reads from a sweet corn panel with 693 genotypes to build a presence/absence (PAV) table based on k-mers and call SNP variants. The population was phenotyped for a variety of traits measured across three years. The k-mer-based pipeline begins performing the k-mer occurrence count (KOC) of 31 bp from the raw sequences for each individual. After the filtering steps, a k-mer presence/absence table was created to represent the genetic diversity in the population. GWAS analysis was conducted using a linear mixed model (LMM) in GEMMA, with k-mers filtered for minor allele frequency (MAF) 0.05. For the SNP-based GWAS approach, we called variants using GATK and obtained a VCF with 16M SNPs. Overall, we found that for some traits, the k-mer-based approach was more effective at identifying significant genomic regions. Additionally, for some traits, the k-mer-based GWAS was more consistent across years of data for the same trait. However, a disadvantage of the approach is that the pipeline is significantly more time-consuming and computational-intensive.

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P210

Comprehensive kernel analysis in large DH populations derived from intercrossing germplasm enhancement of maize lines

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Inadequate absorption of micronutrients, termed “hidden hunger,” impacts more than half of the global population. Those predominately affected rely on staple crops for their caloric intake, which are often low in bioavailability of the micronutrients needed. Iron, zinc, and provitamin A, the predominately lacking micronutrients, have been extensively researched to facilitate biofortification, a plant breeding method that exploits biosynthetic metabolic processes to increase the concentration of nutrient-value components. However, an evaluation for these key value-adding traits has not been thoroughly studied within the Germplasm Enhancement of Maize (GEM) Project germplasm pool. Whereas commercial hybrid varieties have been selected for high-yielding traits, the exotic donor germplasm utilized by GEM has been historically bred for edible traits, thereby increasing the likelihood of discovering impactful variation for introgression into the corn industry. Two doubled haploid (DH) populations were derived from double-double synthetic crosses to encapsulate the genetic diversity within GEM that represent the two major Corn-Belt heterotic groups. DH lines will be genotyped using a low-density genotype-by sequencing strategy, while double-double cross founder lines will be genotyped using high-density genome sequencing. This approach will allow for imputation with improved accuracies. DH lines will be phenotyped for (1) macromolecular composition using near-infrared spectroscopy (NIR), (2) provitamin A carotenoid concentrations using high performance liquid chromatography (HPLC), (3) mineral concentrations using inductively coupled plasma – mass spectrometry (ICP-MS), and (4) antinutrient concentrations for bioavailability assessments. This study will combine genotypic data with phenotypic data in an optimal training populations approach to identify lines superior in value-added traits within GEM.

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P211

Contribution of tropical alleles to heterosis in root traits and root-associated microbial traits under varied nitrogen conditions

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The increased genetic load resulting from the cost of domestication and the limited founding parental lines impedes the continuous improvement of U.S. temperate maize germplasm, particularly in the context of climate change. To enhance genetic diversity and mitigate the genetic load, the USDA-funded Germplasm Enhancement of Maize (GEM) program aims to leverage tropical alleles for enhancing temperate genetic materials. In this study, we conducted a replicated field experiment under low and high N field conditions over three consecutive years to evaluate the effect of tropical alleles of GEM materials in responding to different nitrogen (N) conditions. From the field experiments, we collected conventional phenotypic traits, including six yield-related and seven belowground root traits, as well as rhizosphere microbial traits from a Backcrossed GEM (BGEM) inbred panel and the hybrids generated by crossing BGEM inbreds with two tester lines, B73 and Mo17. Our results reveal that BGEM hybrids consistently outperform inbreds for conventional phenotypic traits under both N conditions. Interestingly, microbial traits generally display lower abundance and diversity in hybrids than inbreds under high N conditions. However, under low N conditions, about half of the microbial traits exhibit heterosis. To uncover the genetic basis of trait per se, heterosis, and the transformed N-responsive traits, we conducted Genome-wide Association Studies (GWAS) and identified a number of favorable tropical alleles, some of which contributed to heterosis of conventional or microbial traits and associated with N-responsive traits. The insights gained from this study can potentially contribute to developing N-resilient maize varieties by leveraging host-microbe symbiosis in future maize breeding.

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

P212

D16 – A game-changer in the quest to generate short-stature corn hybrids

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One way to achieve sustainably high crop yields is by planting cultivars with reduced height. The major benefit of this trait stems largely from the ability of short plants to resist lodging, thereby allowing the adoption of optimal management approaches to maximize yields. The significance of the impact that this trait has had on crop productivity is best exemplified by the development of dwarfing varieties of wheat and rice in the late '60s, which led to an era of food sufficiency, commonly referred to as Green Revolution. Since then, the dwarfing trait has become a staple for most cereal crops. However, there has been one gaping exception. Corn! While it was neither due to the lack of intent nor effort to exploit this trait in corn, dwarfing failed to be tamed to generate commercially viable hybrids. The reason: it is not as straightforward as one may think to develop short cultivars in a hybrid crop. A major bottleneck is the lack of a suitable dwarfing trait that should preferably be dominant with negligible impacts of genetic background and heterosis. That said, it is exciting to announce that we have finally found one such mutant that has the potential to transform maize production worldwide. Named *D16*, it was generated by chemical mutagenesis with EMS. *D16* is partially dominant, and it compresses plant height by 2-3 feet in all corn hybrids tested. The impact on yield appears to be positive also. *D16* is caused by a unique point mutation that had never been reported in any plant species. *D16* can be bred in maize by both conventional and gene editing (GE) approaches. The advantage of GE is that it will allow the *D16* trait to be extended to other crops as well.

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P213

Developing transformable haploid inducers to meet commercial-scale genome editing in maize

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Introducing favorable alleles into commercial crop germplasm is time consuming and costly. Two technologies that are disrupting maize breeding are doubled haploids (DH) and genome editing (GE). Recently, DH and GE were combined into HI-Edit ™, a method in which a haploid inducer (HI) line is transformed with a CRISPR cassette to deliver an edit to any maize variety in a single cross, obviating recurrent selection and linkage drag from trait introgression. HI can be achieved through maternal HI system triggered by mutations of MTL/ZmPLA1/NLD, and paternal HI system through engineered CENTROMERIC HISTONE3 (CENH3). Typically, HI lines are less amenable to transformation given their aberrant reproductive characteristics. For HI-Edit ™ to operate at commercial scale, there is an urgent need to develop an efficient and transformable HI lines. Leveraging marker assisted selection for HI genes (matl, dmp, R1, and C1) and four generations of phenotyping, we developed maternal *MATL* HI lines in Iodent and Stiff Stalk germplasm with a combination of high transformability, HI performance, and a dominant anthocyanin embryo marker for haploid identification. In addition, a new paternal *CenH3* HI line bred with color marker was developed in Stiff Stalk germplasm showing desirable performances in transformation frequency and HI. These results may facilitate the development of commercially scalable HI-Edit™ for maize and other crops.

P214

Discovering phosphorus efficiency alleles in maize and sorghum by combining multiple omics datasets and advanced modeling statistics

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Phosphorus(P) is an important nutrient in fertilizers which is essential for plant growth and development. Excessive use of P-rich fertilizers leads to leaching and runoff to water bodies harming aquatic life. The limited global reserves of rock P and water pollution make it necessary to find a sustainable solution that ensures the proper utilization of P. Landrace varieties adapted in soils with varying P levels likely possess unique genetic mechanisms to cope with P scarcity. Environmental Genome-Wide Association Studies(eGWAS) using these genotypes with georeferenced accessions, present a potential for identifying candidate genes. Employing eGWAS, we seek to identify candidates associated with P that overcomes these obstacles. Central to GWAS's success is accurate phenotype measurement. To this end, we have developed a Random Forest model predicting P availability surpassing current models in prediction accuracy and capability to discern lower-end values. Utilizing genetic datasets of maize and sorghum, we will conduct eGWAS using our new P data. The maize dataset specifically has gone through Practical Haplotype Graph imputations, increasing its resolution. We will use a linear regression based p-value combination method to aggregate multiple small effects on a gene-based level. This study will also investigate the role of lipids in sorghum's adaptation to P levels in Africa, drawing comparisons with our previous study that identified lipid variations in maize adapted to low P in the Mexican highlands. Additionally, we have obtained lipid profiles for 400 Sorghum Association Panel grown in high and low P. Analyzing their lipid dynamics will play a significant role in understanding P utilization. Subsequently, a GWAS will be conducted focusing on these identified candidate lipids. Using the Cauchy Combination test to combine the findings from both the P GWAS and lipid GWAS, we aim to redefine the order of gene importance and identify candidates linked to both phenotypes.

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P215

Dissecting the genetic architecture of root traits in the MEXIMAGIC population

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Within Mexican territory alone, the tireless efforts of generations of farmers have produced 50+ recognized varieties of native maize, adapted to elevations from sea level to well over 3,000 m and environments ranging from semi-desert to the hot, humid tropics. As maize dispersed, it was the roots that most directly faced many challenges posed by new environments. While the diversity of ear morphology readily illustrates the richness of Mexican maize, below-ground traits may be of greatest importance in developing maize varieties for future climates. Here, we present high-throughput root phenotyping approaches that are beginning to provide a picture of the variation in root system architecture and root anatomy to be found in Mexican native maize. We introduce a novel multi-parent advanced generation inter-cross mapping population for Mexican native maize (MEXIMAGIC) and illustrate its application in testing adaptive hypotheses. Following the development of test cross-families developed using eight parent families, the root systems of 169 F1 families were evaluated in a common garden field experiment, allowing for the identification of Quantitative Trait Loci (QTL) for root system width, nodal root number, and lateral root density. Parsing QTL alleles to their respective founders reveals substantial variation in the parental allele effects, displaying diverse phenotypes. Further analysis of these allele effects paired with climate variables describing the native climate of the eight founders allows for the additional identification of putative signals of local adaptation. These adaptive QTL present building blocks for developing maize varieties displaying root systems conferring resistance to abiotic stresses. With more than 500 families, continued use of the MEXIMAGIC population will provide substantial power to identify QTL at a higher resolution. Further evaluations in multiple soil environments will provide additional information into the adaptive potential of parental alleles responsible for root traits conferring resilience in the face of climate change.

P216

Effects of maize chlorotic mottle virus and potyvirus resistance on maize lethal necrosis disease

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Maize lethal necrosis (MLN) is a viral disease caused by host co-infection by maize chlorotic mottle virus (MCMV) and a potyvirus, such as sugarcane mosaic virus (SCMV). This disease is most effectively managed by growing MLN resistant varieties, although the relative importance of MCMV versus potyvirus resistance in managing this synergistic disease is poorly characterized. Here, we explore the impact of host resistance to SCMV and/or MCMV infection on plant disease, virus titer, and synergism during co-infection. In addition to visual assessment of disease progress, we used qRT-PCR to quantify relative virus titer and expression of known potyvirus resistance genes *TrxH* and *ABP1*. MLN disease was significantly lower in both the MCMV resistant (N211, CML333, and KS23-6) and SCMV resistant (Pa405) inbred lines, compared to the susceptible control Oh28. Only Pa405 sustained resistance to SCMV in both treatments, though both CML333 and KS23-6 had reduced SCMV titer throughout MLN infection. Despite no visible presence of disease, MCMV titers in N211 were not significantly different from susceptible controls by 14 days post inoculation. Initial *TrxH* expression was up to 49,000-fold lower in Oh28 compared to other genotypes, while expression of *ABP1* was up to 4.5-fold lower. Measures of virus synergy indicate that while MCMV resistance is effective in early infection, strong potyvirus resistance is critical for reducing synergist effects of co-infection on MCMV titer. These results emphasize the importance of both potyvirus resistance and MCMV resistance in an effective breeding program for MLN management.

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P217

Effects of plot orientation and leaf angle on maize grain yield

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Increasing the number of plants per acre has been an effective means for achieving higher overall maize grain yield, but planting at densities above certain thresholds can have detrimental effects on maize plants, including ear barrenness and increased stalk and root lodging risk. Furthermore, altering planting density adds costs to producers via greater expenditures on seeds and fertilizer to attain target planting densities and satisfy higher demand for nutrients, ultimately reducing the return on investment. Efforts are needed to identify effective methods for increasing maize grain yield after maximum planting rates have been reached by increasing yield per plant. We hypothesize that maximizing light interception through optimum combinations of canopy architecture and plot orientation is one such method. Here, we utilized a split-plot design to investigate twenty maize testcross hybrids with distinct leaf angle architectures (flat, upright, or dynamic), with two replications of two-row plots at two locations in Iowa, USA. At each location, plots were planted either parallel or perpendicular to the general position of the sun. Data were collected for root and stalk lodging, ear barrenness, light interception forty days after planting and at flowering, multiple leaf angles per canopy type, grain moisture, and grain yield. Our findings indicate altering plot orientation does affect light interception, an important determinant of maize grain yield. Identifying leaf angle and plot orientation combinations that optimize light interception may provide producers with a means for increasing overall maize grain yield.

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P218 @Tobsy7659

Examining the dynamics of microbe-driven heterosis in maize

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Heterosis, or hybrid vigor, is a pivotal factor in modern agriculture, significantly boosting crop yields. While extensive research has delved into genetic and environmental influences on heterosis, the microbial role remains poorly understood. This study investigates the intricate relationship between maize plants and their associated microbial communities, unveiling the microbial impact on heterosis. Our approach involved using a simplified synthetic community (SynCom) of seven bacterial species assembled by maize plants. We evaluated the individual and collective capacity of each bacterial species to induce heterosis across four maize genotypes—two inbreds (B73 and Mo17) and their hybrids (B73 x Mo17 and Mo17 x B73). To achieve this, surface-sterilised maize seeds were pregerminated and inoculated with individual bacterial species or SynCom and grown gnotobiotically. Results revealed that heterosis was more pronounced in the shoots and roots of the uninoculated control, while it manifested solely in the roots of the SynCom treatment. Moreover, heterosis effects in both shoots and roots were more influenced by some individual microbes within the SynCom than by others. Future experiments aim to establish multiple iterations between heterosis inducers and non-inducers, exploring potential microbe-microbe influences on maize plant heterosis. This research not only broadens our comprehension of plant-microbe interactions but also holds significant promise for leveraging microbial communities to sustainably optimize maize productivity.

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

P219

Exploring the application of phenomic selection in corn breeding.

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Phenomic Selection (PS) is a low-cost alternative to Genomic Selection (GS) for enhancing genetic gains in breeding programs. That new technique maintains the statistical procedure used in GS-based prediction models but replaces the molecular markers data (*e.g.* SNP data) with variables obtained from a multi-variate phenotyping method (*e.g.* near-infrared spectroscopy (NIRS) data). This study aimed to explore the application of PS using single kernel NIR in a sweet corn breeding program, focusing on predicting field-based traits of economic importance, including ear and vegetative characteristics. First, on a diversity panel, three models were employed: G-BLUP and NIRS BLUP models, which utilized relationship matrices based on SNP and NIRS data, respectively, and a third model that uses both matrices as independent terms. The genomic relationship matrices were evaluated when the number of SNPs used to built the matrix varied from 500 to 200,000 SNPs. The objective of this test was to evaluate if PS can complement GS applications under low marker densities. In a second approach, we utilized the NIRS BLUP model to select doubled haploid (DH) lines for germination before planting. Our findings reveal that while G-BLUP models generally outperformed NIRS BLUP models, the model combining both information (PS+GS) yielded the highest accuracy. The increase of the combined model relative to GS alone was particularly higher when low marker densities were used. This indicates NIRS's potential to maintain/improve accuracy together with SNP-based information while reducing marker density, which could decrease genotyping cost in the breeding program. Additionally, results showed that the NIRS BLUP model alone effectively distinguished between high and low germination rates in DH lines, with an accuracy of 0.71. In conclusion, PS is a promising low-cost tool that could help to optimize the sweet corn breeding program.

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

P220

Expression analysis of key genes controlling resistance to *Striga hermonthica* in tropical maize inbred lines

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A severe infestation of Striga hermonthica dramatically reduces maize yield in Africa. Resistance to Striga is an essential trait in a new maize cultivar developed for release. The development of predictive markers is a prerequisite to expedite the genetic improvement of maize. Expression analysis based on RNAseq was performed to study the transcriptome profile of susceptible (5057) and tolerant (ZD05) maize genotypes grown in rhizotron, with and without infestation. Root tissues were collected in three replications at 3, 9, and 22 days post-infestation (dpi). The transcripts were compared to identify the up-and down-regulated genes for each genotype at each time point using a log2 fold-change of 1.5 as a threshold. Functional annotation and pathway enrichment analysis identified differentially expressed genes (DEGs). Based on the current data and literature review, eight DEGs were selected for validation of their expression with real-time quantitative reverse transcription polymerase chain reaction (RT-qPCR). The result of the qPCR experiment was analyzed using the E-method ratio of the Advanced Relative Quantification Experiment with an external reference in the LightCycler 480 software. The relative quantification technique compares the levels of two different target sequences in a single sample. The relative expression of all target genes agrees with the previous transcriptomic results, with an inverse relationship between respective genes' expression in resistant and susceptible genotypes. The study corroborated that the resistant inbred line supports a significantly lower level of *Striga* development. Moreover, the resistant (ZD05) genotype up-regulates more genes involved in plant secondary metabolism, defence and cellular transport. The initial results suggest that the downregulation of host genes seems essential for establishing parasitic weed infestation. Expression analysis revealed key genes providing an insight into the mechanism of host-plant resistance. These putative/candidate genes can be utilized in developing robust trait-linked markers for marker-aided selection of Striga-resistant germplasm.

Funding acknowledgement: Bill & Melinda Gates Foundation

P221 @SilvioSalvi2

Fine mapping and positional cloning of a major root system architecture QTL in maize

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Root system architecture (RSA) crucially affects soil resources utilization, including water absorption. Hence, comprehensive understanding of the genetic and physiological processes underlying RSA development is critical in order to inform breeding programs aimed at improving resilience to abiotic stresses. In this study, we characterized and cloned qRoot-yield-1.06, a major QTL associated to maize RSA traits and yield stability over water regimes. qRoot-yield-1.06 was previously mapped on bin 1.06 in the cross between Lo964 and Lo1016, two inbred lines known to differ in their RSA. The QTL effect was confirmed by developing pairs of near-isogenic lines which solely differed in the allelic constitution of the target QTL. QTL fine mapping and positional cloning were carried out exploiting a large nearly-isogenic recombinant population. Root phenotyping was based on shovelomics combined with software-assisted root images analysis. A list of candidate genes was prioritized and the causative gene, a SOS-like Na+/H+ antiporter, was identified using a combination of approaches including TILLING, qRT-PCR and transcriptomics. We showed that the (+) QTL allele from Lo1016 enhances the root system size by increasing in the number and length of lateral roots, in comparison with the (-) QTL allele from Lo964. Our findings indicated that native allele sequence variation at a SOS-like gene impacts RSA under non-saline conditions, suggesting a role of this gene in root development.

P222 @divine_hein

Functional genetics of G×E QTL for root system architecture in maize

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Complex phenotypes are influenced by genetic variation, environmental differences, and genotype-by-environment interactions (G×E). We used field experiments with two levels of the environmental factor soil moisture to identify constitutive and G×E QTL in several mapping populations in maize. To gain a better understanding of gene function and phenotypic effect of these QTL (Woods et al., 2022), we used a functional genetics approach to perturb individual gene models. For each gene model, we searched for loss-of-function mutations within the coding sequence, generated by the insertion of UniformMu transposable elements (McCarty et al., 2005). We created BCF3 lines in order to be able to evaluate root phenotypes of genotypes with alternate homozygous alleles at the target gene models. Measurement of root pulling force (RPF) on plants at silking showed that the mutant line had significantly higher RPF than the wild-type ($p < 0.05$). The variation in RPF that we observed captured differences in other feature-extracted root traits such as area, depth, and number of root tips. Building on the field-based observations, our hypothesis posits that alternative states at this gene lead to varying signals of nutrient deprivation and supplementation, subsequently influencing distinct patterns of root growth. We aim to characterize and compare gene function, evaluating its impact on root trait variation within mutant and wild-type lines subjected to two levels of factor soil moisture in a growth chamber experiment. Additional functional genetics will be addressed to provide cross-species insights, thereby enhancing our biological knowledge of the genetic architecture that regulates complex root traits. McCarty DR, Settles AM, Suzuki M, Tan BC, S Latshaw, et al. (2005) Steady-state transposon mutagenesis in inbred maize. Plant J 44: 52–61. Woods P, Lehner KR, Hein K, Mullen JL, McKay JK (2022) Root pulling force across drought in maize reveals genotype by environment interactions and candidate genes. Frontiers in Plant Science 13:883209. https://doi.org/10.3389/fpls.2022.883209

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P223

GWAS analysis reveals resistant genes to maize southern leaf blight

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Southern leaf blight (SLB), caused by the necrotrophic fungal pathogen *Cochliobolus heterostrophus*, is a major foliar disease of maize worldwide. Identifying genetic variations contributing to resistance against SLB is crucial for maize yield and quality. 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. Overall, fifteen significant variants corresponding to ten candidate genes were identified as associated with SLB resistance. We found thirteen of the fifteen associated SNPs co-localized with previously reported quantitative trait loci (QTLs). Two annotated genes located in the SLB-resistant QTLs were shown to be involved in plant defense pathways. Through insertional mutagenesis and transgenic validation, we demonstrated that *ZmMAPKKK45*, which encodes a mitogen activated protein (MAP) kinase kinase kinase (MAPKKK) associated with the MAPK cascade, is positively regulating SLB resistance. Overexpression of another candidate gene *ZmLSP*, which encodes a leaf senescence-related protein could significantly enhance the resistance of maize to SLB. Our results provide resistant loci/genes as direct targets for crop resistance genetic improvement.

Gene / Gene Models described: *mapkkk45*; GRMZM2G080499
Funding acknowledgement: National Science Foundation (NSF)

P224

Genetic determinants of aerial root formation in maize: Prospects for harnessing biological nitrogen fixation

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Indigenous maize (*Zea mays*) varieties from the Oaxaca region of Mexico have been shown to support nitrogen-fixing bacteria in mucilage produced by their aerial roots. Maize plants with this trait can obtain 29-82% of their nitrogen from this symbiosis in the right environmental conditions. A better understanding of the genetic factors that contribute to this trait will enable greater use of biological nitrogen fixation to support the global demand for cereals and reduce dependence on synthetic nitrogen fertilizers. Here we assess population structure among eight populations of doubled haploids generated from inbred PHZ51 and three landrace parents. A two-location field trial of two doubled haploid populations shows heritability is generally low for the number of nodes with aerial roots, the number of roots per node, and the root diameter. However, noticeable differences are observed between populations that are consistent across locations. Furthermore, we identify QTL associated with aerial root traits unique to each population and shared between populations. While additional research is needed to confirm and expand these results, our findings support pursuing the development and adoption of maize varieties with this trait to improve global food insecurity and reduce environmental degradation attributed to synthetic fertilizer use.

Funding acknowledgement: United States Department of Agriculture (USDA)

P225

Genetic potential of tropical, temperate, and mixed maize populations evaluated in tropical environments

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The introduction of exotic germplasm in a breeding program is a pre-requisite to broaden the genetic basis of germplasm breeding. Recurrent selection is an important breeding scheme for populations adaptation. The main proposal of our work was to evaluate the genetic potential of the 16 tropical, mixed, and temperate germplasm populations across tropical environments. The 16 populations were composed of tropical, temperate, and mixed maize populations structured in half sibs and progenies S1 evaluated across two locations at Minas Gerais, Brazil. A mixed model approach was used to estimate variance components, and genetic gains of the 20 progenies for recombining were predicted based on grain yield (GY) per se and multiple traits FAI-BLUP (Factor Analysis and Genotype-ideotype Design) index. Selection was done to reduce flowering time (days to silking - DTS) and increase GY. Also, selection was performed based on each local and across locals. Our results showed that there was large genotypic variation for all trials. Variance components due to progenies were highly significant (*P0.10*) for all trials. The estimates of broad-sense heritability were high and ranged from 0.64 (UFVM200(HS)C3) to 0.87 (BS17). Although genetic predicted gains were high for per se GY selection, the index selection provided more efficient predicted gains for architecture and flowering traits. Therefore, we decided to use the index selection. For populations BR106, BRSSM, BS16, BS26, BS27, BS28, BS29, FS8A(S), FS8B(S) and UFVM100(HS)C2, selection for each environment was more successful, while for BR105(S)C1, BS17, BSTL, IPR164 and UFVM200(HS)C3, selection based on average of locals were better. We concluded that there is genetic variability within the populations, so it is possible to select, and that selection based on index were the best strategy.

Funding acknowledgement: United States Department of Agriculture (USDA), CAPES, CNPq, FAPEMIG

P226

Genetic analysis of yield, combining ability, and agronomic performance in non-Stiff Stalk elite germplasm in a factorial mapping population

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A nested factorial mapping population of 1,959 doubled-haploid inbreds across twelve bi-parental populations was used to characterize yield and agronomic performance in the non-Stiff Stalk heterotic pattern. Six parental lines of this population are expired Plant Variety Protection inbreds, which have an important role in the core germplasm of modern proprietary germplasm. The last parent is a public line derived from material released by the Germplasm Enhancement of Maize project. Two hybrid sets were generated using unrelated Stiff Stalk testers as males, PHT69 and FBLL. This study aims to identify Quantitative Trait Loci (QTL) in elite germplasm under selection for complementary effects and their implications for traits of interest. A genetic analysis was conducted using a multi-parent QTL structure on a multi-environment yield trial grown in 2017, 2018, and 2019. We found a weak correlation between female inbred performance in the two testers, indicating a nonadditive effect for grain yield. Significant QTLs that were specific to testers, across testers, and the difference between testers for various complex traits, such as anthesis and silking, grain moisture, test weight, and grain yield, were identified. Parental allelic effects from the twelve female populations were tester-specific, highlighting the importance of complementarity in hybrid performance.

P227 @HongyuJin5

Genetic elucidation of maize leaf length and width traits

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Canopy architecture plays a key role in determining photosynthetic productivity, efficiency, and ultimately biomass and grain yield. Canopy architecture is, in turn, determined by combination of agronomic decisions, plant spacing and layout, and plant phenotypes, including leaf number, leaf angle, leaf length, and leaf width. While a number of studies has focused on identifying genes involved determining leaf number – often the result of changes in flowering time – and changes in leaf angle, the determinants of variation in leaf length and leaf width have been comparatively less investigated. Here we developed and employed a pipeline to conduct resampling based GWAS with BLINK and used it to identify genomic intervals linked to variation in leaf length and leaf width in a dataset collected from the Wisconsin Diversity panel over two field seasons. Taking advantage of a new and higher density genetic marker dataset derived from whole genome resequencing, we identified a total of 9 loci significantly associated with leaf length and an additional 9 loci associated with leaf width. None of them overlapped. We evaluated the repeatability and consistency of these loci using data from the SAM association panel collected from a third environment. Ten of the 18 originally identified loci showed significant effects on the same loci in this replication study. Change in allele frequency for the loci identified in this study among maize populations from different continents suggest some of these loci altering maize leaf morphology may have been targets of direct or indirect selection during the adaption of maize to different parts of the globe.

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

P228

Genetic map-based cloning of two major QTL genes for husk number in maize (*Zea Mays*)

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The maize husk provides a suitable environment to protect the early stage of ear growth and development. Reducing the husk leaf number (HLN) properly is one of the main goals in maize breeding to develop new varieties suitable for mechanical harvesting. HLN is a complex quantitative trait which genetic basis remains unclear so far. We investigated HLN on more than 400 maize inbred lines and constructed segregation populations. The HLN QTL genes was mapped and further fine mapped by using recombinant-derived progeny strategy. Two major QTLs *qHLN1* and *qHLN6* were finally narrowed down and located on chromosome 1 and 6, respectively. *qHLN1* was located in the 58Kb physical region, in which only one gene encoding a transcription factor was named *ZmHLN1*. Through qRT-PCR analysis of NIL-$qHLN1^{BS351}$ and NIL-$qHLN1^{US043}$, and EMS mutant with early termination of *ZmHLN1* led to more husk leaf number compared with wild type. *ZmHLN1* was identified as the target gene controlling husk number. *qHLN6* is mapped to 1.2-Kb region in the promoter of *ZmHLN6*, which also encodes a transcription factor. $qHLN6^{US043}$ decreased 2~3 husk leaves relative to $qHLN6^{BS351}$. Two independent mutant lines consistently increase ~10 more husk leaves. qRT-PCR analysis confirmed that *ZmHLN6* was highly expressed in NIL-$qHLN6^{US043}$ relative to NIL-$qHLN6^{BS351}$. Thus, the 1.2-Kb region in the promoter of *ZmHLN6* should contain functional motifs in HLN. Furthermore, the yeast two-hybrid assay (Y2H) and the split firefly luciferase complementation assay in tobacco leaf epidermal cells have been conducted. Both assays showed that *ZmHLN1* and *ZmHLN6* physically interact in vitro and in vivo. Introgression of $qHLN1^{US043}$ and $qHLN6^{US043}$$^{\wedge\wedge\wedge}$ into modern maize inbreds and hybrids significantly decreased HLN without affecting yield. The analyses of natural variations and domestication characteristics for two genes will be performed to develop new molecular markers for molecular breeding in Maize.

Gene / Gene Models described: *HLN1,HLN6*; no
Funding acknowledgement: National Science Foundation China

P229 @olamide_olamz

Genetic mapping of resistance to Goss's wilt of maize

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Goss's bacterial wilt and leaf blight, caused by the Gram-positive actinobacterium *Clavibacter nebraskensis* (Cn), is a significant bacterial disease of maize and affects the maize production in the US and Canada. Similar to many plant pathogens, genetic resistance remains one of the best control strategies. However, host responses to Gram-positive bacteria and the molecular and genetic resistance mechanisms associated with developing plant disease symptoms caused by Cn in maize are poorly understood. We analyzed gene expression upon Cn infection in both resistant (R) and susceptible (S) lines, identifying Cn-triggering expression of genes involved in multiple hormone pathways. The mutants of some of those genes are being collected from the public stock center or generated through CRISPR editing for genetic analysis. At the same time, mapping of the resistance gene has identified multiple genomic regions conferring Goss's wilt resistance. We have constructed mapping populations for further finer-scale mapping. In this poster, we will update our mapping result through using Bulk Segregant RNA-seq (BSR-seq). Our integrated approach aims to provide foundational data for mechanistic studies to mitigate the effects of this important maize disease.

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P230

Genome-wide dissection of leaf angle variation across the canopy in maize

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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.

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P231

Genomic selection: Essence, applications and prospects

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Genomic selection (GS) emerged as part of the solution to ensure food supply for the growing human population, thanks to advances in genotyping technologies and improved understanding of quantitative genetics and the genotype-phenotype relationship. In essence, GS is a breeding strategy to predict the genetic merits of individual genotypes for selection using their genotypic data and a trained model. It includes three major steps: training population design, model building, and prediction and selection. With GS, the role of phenotyping in breeding has switched from generating data for selection to generating data for model building. The increased capacity of GS to evaluate more genotypes, in combination with shorter breeding cycles, has led to a wider adoption in plant breeding. Many research studies have been conducted to optimize GS with greater emphases on crop- and trait-specific applications, and predictive models. A successful GS strategy, however, encompasses more than just predictive models; it also involves strategic design and planning. The most significant advances in GS include integrating phenomics, turbocharging gene banks, analyzing multitrait and longitudinal traits, and crosslinking crop growth models, environmental covariates, and reaction norms. In light of the rapid development of artificial intelligence (AI), GS can be further improved by either upgrading the entire framework or individual components. AI-driven GS can consider new layers of information including multi-omic, enviromic, and pangenome datasets. As GS complexity increases, so do the challenges associated with storing, handling, and processing large volumes of data, as well as the need for higher expertise across various domains. Technology advances, research innovations, and emerging challenges in agriculture will continue to shape the role of GS in plant breeding.

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P232

Genomic prediction and landscape genomics in a large maize landraces collection using high-throughput pool genotyping identifies promising sources of diversity for prebreeding

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Maize landraces are a valuable source of genetic diversity for facing climate change due to their local adaptation. High-throughput pool genotyping (HPG) is a cost-effective approach to genotype maize landraces and identify promising sources of alleles for tolerance to abiotic stress. We applied this approach on a large collection of European and American maize landraces to i) characterize its genetic structuration; ii) identify genomic regions involved in adaptation through environmental association studies; iii) perform genomic prediction (GP) of both adaptive and agronomic traits. Landraces were structured according to their history and environmental conditions. GP yielded high accuracy, allowing to identify promising landraces. We identified genomic regions associated with bioclimatic variables that could be putatively involved in adaptation to abiotic stress. Combining eco-genetic and genomic prediction opens an avenue for using these genetic resources for prebreeding.

Funding acknowledgement: EVA-ECPGR, Amaizing, Promais, INRAE, CIMMYT, MineLandDiv

P233

High intensity phenotyping sites: multi-scale, multi-modal sensing to identify the genetic regulation of plasticity/phenotypic stability.

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In a world with a changing climate, the yield stability of crops has become a major concern. To better understand the genetic control of yield stability, crop traits and environmental data were collected from common sets of maize hybrids (including a new set of era hybrids, ex-PVP hybrids, and commercial hybrids) as well as the SAM diversity panel which consists of ~350 inbreds were grown over three years (2021-2023) at six locations from western Nebraska to eastern Iowa that span elevation and annual rainfall gradients. At some locations multiple levels of N fertilizer and/or irrigation were applied. In total, this project assayed crop performance in 47 distinct environments. Hybrids were grown in replicated four-row yield plots, while the inbreds were grown in replicated two-row plots. Multiple types of trait data (e.g., plant and ear height, days to anthesis, a variety of ear traits, stalk nitrate concentration, and transpiration) and environmental data were collected using traditional manual assays, robots, UAVs, satellites, and novel sensors. The resulting data are being analyzed to identify genes associated with plasticity/stability of multiple traits.

Funding acknowledgement: United States Department of Agriculture (USDA)

P234

High-throughput phenotyping and machine-learning based approaches for time-series crop assessment using UAV imagery

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Modern breeding programs rely heavily on efficient methods to screen large numbers of diverse genotypes for traits of interest, such as disease resistance, drought tolerance, or improved yield. After identifying the genetic loci or genes that associate with these traits using GWAS or functional analyses, it will then benefit future plant breeding efforts seeking to incorporate these traits into new crop varieties, whether through conventional breeding or gene editing techniques. Unmanned aerial vehicle (UAV)-based image data has been increasingly used for this task, as it allows entire test plots to be quickly and cost effectively phenotyped. Previously, we have developed methods to improve accuracy of machine learning techniques for automated tassel counting by removing non-tassel pixels, as well as identify vegetation indices correlated with traits like leaf area, nitrogen (N) content, and kernel weight and then use GWAS to identify genes associated with these UAV-extracted traits. In this study, we used both multispectral and visible color (RGB) image data to develop N-responsive vegetation indices (VI). This enables UAV-based imagery to be used as a reliable predictor of N-responsive traits, and can be leveraged to track plant growth stages and health through the season. Eventually, we will incorporate the N-responsive VIs into the genomic selection pipeline to develop N-resilient maize.

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

P235

Host-specificities of microbial consortia revealed by metagenomic approaches in *Sorghum bicolor*

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Plant-microbiome interactions play important roles in mediating plant responses to ever changing environmental conditions. Arbuscular mycorrhizal fungi (AMF) are key symbionts of many plants and can provide benefits to their host such as enhanced nutrient uptake and drought tolerance. Close AMF-bacterial interactions may also make a historically underappreciated contribution to the symbiosis through mechanisms such as nutrient ion release from the surrounding soil. Understanding this tripartite interaction will allow us to develop strategies for maximizing microbiome benefits to bioenergy crops such as sorghum that are grown with few inputs on marginal land to avoid competition with food crops. Through multi-year field trials across contrasting ecoregions in the United States (GA and AZ), we seek to identify genotypes that can best benefit from AMF associations, and sorghum genetic markers related both to the symbiosis and specific partner choice. Here, we present results from the ongoing analysis of the first year GWAS study in GA where we implemented a factorial N/P fertilization experiment for 337 sorghum genotypes from a bioenergy association panel. Through this, we documented the extent of variation in both aboveground yield phenotypes and the AMF species recruited by individual genotypes across the panel. We also have used this system to explore methods of how to best identify and quantify the composition and abundance of bacterial consortia within plant roots. On a subset of samples from the factorial fertilization treatments, we implemented both amplicon and whole-genome shotgun sequence approaches to develop more accurate quantitation methods of microbial abundances based on host-plant sequence normalization. Through this approach, we reveal patterns in bacterial composition not apparent from traditional analysis alone.

Funding acknowledgement: Department of Energy (DOE)

P236

Identification of genomic regions associated with the causal QTL of SHDG trait in Ames diversity panel by GWAS

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The doubled haploid (DH) approach is a promising alternative to traditional self-pollination for inbred line development, primarily because it decreases the time taken to obtain homozygous lines. Currently, DH technology is widely dependent on chemical treatment (colchicine) to induce haploid genome doubling and, subsequently, male fertility. These chemicals can be harmful to humans and plants and have typically recorded a doubling rate of about 10 to 30% due to the recalcitrance of some temperate genetic materials. Thus, genome doubling remains a bottleneck to large-scale DH production. Although to a limited extent, spontaneous haploid genome doubling (SHGD) and male fertility of maize haploids (without chemical treatment) have been studied and recorded doubling rates ranging from less than 5% to greater than 50%. Recently, there has been increased interest to omit chemical treatment and rely on SHGD as it would eliminate the use of colchicine for genome doubling and make it possible to directly plant a haploid seed in the field and avoid greenhouse costs. A major breakthrough was the discovery of a maize line A427 with SHGD and a major QTL mapped to chromosome 5. The introgression of this major QTL to elite germplasm may overcome the need to use colchicine. However, it is necessary to identify additional sources of major QTL for SHGD in maize as they may (1) simplify managing SHGD in different heterotic groups and (2) allow simplified introgression procedures into elite germplasm by stacking respective SHGD QTL. This genome-wide association study (GWAS) aims to identify additional sources of major QTL controlling SHGD.

Funding acknowledgement: United States Department of Agriculture (USDA)

P237

Identification of waxy genotype from iodine-stained maize pollen image by linear discriminant analysis

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Waxy maize is used as industrial raw material, feed, and food. It carries the recessive *waxy1 (wx1)* gene that elevates amylopectin content to 95-100% compared to 70-75% in normal counterpart. Iodine staining for maize pollen could serve as a straightforward and cost-effective method for waxy genotype. Here, we describe a method for rapid identification of waxy genotypes by RGB values from stained pollen images and linear discriminant analysis (LDA). A set of F2 segregating population were planted to identify genotype of individual plant along with two parental lines and F1 plants serving as known genotype information for LDA modelling. Pollens of wild type *(Wx1Wx1)* stained into black color, while pollens of waxy type *(wx1wx1)* remained unstained displaying yellow or brown color. Heterozygous type *(Wx1wx1)* showed the mixture of stained and non-stained pollens with the ratio of 1:1. Image of stained pollens was captured under a microscope. Individual pollen grains in a captured image from a single plant were determined by setting threshold and the Region of Interest feature in ImageJ software. RGB values from identified pollen grains was extracted and then averaged to obtain a datapoint for the individual plant. Datapoints from two parental lines and F1 plants were used to create a discriminant function in LDA while the function was applied to those from F2 individuals for genotype classification. The LDA results were validated from self-pollinated or test-crossed ears from the F2 individuals. This technique is rapid, accurate and cost effective in identifying waxy genotype in maize and can be easily employed even where marker-assisted breeding is limited.

Gene / Gene Models described: *wx1*; Zm00001eb378140
Funding acknowledgement: Chungbuk National University(CBNU)

P238

Incorporating gene network information into the prediction of maize flowering time

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The flowering time in crops is a critical trait for consistent agricultural performance and developing lines for specific environments. Due to the complex quantitative genetic architecture of flowering time, prediction of diverse lines in new environments is difficult. Much of this complexity arises from the large network of genes and regulatory elements that interact to hasten or slow the transition from vegetative to reproductive growth in the developing maize plants. These interactions, detected through either functional genetics studies with knockout mutants or quantitative genetics studies with natural genetic diversity, contain information critical to understanding flowering time differences between different maize lines. Using gene network information in a predictive context will shift prediction methods towards a systems biology approach, where individual alleles cannot be considered separately from the gene network and genetic background. Although including expression data from over 50 genes in a single model, for example a static mixed-effect model or a dynamic gene regulatory network model, may not be feasible, it is important to consider that in individual maize lines, a unique subset of genes within the network may be able to capture a majority of the trait variation and be sufficient for accurate prediction of flowering time. Simplified gene networks, based only on genes segregating in the genetic background of interest, can incorporate gene network information into modeling approaches without requiring extremely complicated, comprehensive models. Incorporating gene network information within prediction models will also increase the interpretability of results by connecting to functional processes at the cellular level. Genes involved in the flowering time network have diverse functions, including light receptors, transcription factors, and enhancer elements, and the significance of each of these categories in the prediction step can give insight into the biological consequences of gene interactions. The tropical inbred lines CML277 and Tzi8 are given as examples of how network information can be used in predicting the flowering time of maize lines significantly different than the standard reference line B73.

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

P239

Introgression of Zea diploperennis to transfer perennial-like root N remobilization to maize (Zea mays)

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Maize is one of the largest produced crops and a heavy nitrogen feeder and although it is more nitrogen efficient, excess nitrogen fertilizer application and production has proven to be correlated to increased greenhouse gas emissions for either mineral or organic fertilizers. This leads to contamination of underground water bodies and accounts for 6% of US greenhouse gas emissions that is 55% of agriculture emissions. This figure is however low in Africa but is expected to increase by 2050 due to increasing populations in the region and the need to increase food production. In contrast, Africa needs to increase nitrogen fertilizer inputs by 4-fold, but building healthier soils with higher carbon and nitrogen balance could be key as there is evidence that certain root nitrogen remobilization mechanisms are present in perennial relatives of maize. This research seeks to achieve the remobilization of at least 40% of the nitrogen contained in the upper biomass (stover) to the roots late season to increase the organic nitrogen pool. We will therefore be involved in identifying the genetics involved in the remobilization process by backcrossing an inbred line B73 with Zea diploperennis and tracking nitrogen seasonally. We will then genotype the introgressions to identify their positions within the genome. Complementing these studies, we intend to graft Zea diploperennis with Zea mays roots. We hypothesize that grafting perennial roots to the annual shoot system for gene identification purposes will speed gene identification. Assessment will be done on their stay-green properties and their gene expression profiles analyzed. We hope that results from this study can be used to model the impact of future nitrogen recycling varieties for African agriculture.

Funding acknowledgement: United States Department of Agriculture (USDA)

P240

Kernel morphology influences maize hybrid seed production efficiency

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Inbred seed parent production traits, including kernel grade size and the resultant fraction of sellable kernels, impact the commercial feasibility of producing large quantities of hybrid seed. Applying selection for seed production traits during the breeding process enables identification of new inbred lines that will support efficient hybrid seed production. We evaluated a set of 421 inbred lines primarily comprised of materials with Expired Plant Variety Protection (exPVP) for kernel grade size and sellable kernels using manual measurements. The kernels were harvested in 2022 from a replicated experiment grown at the West Madison Agricultural Research Station. The percent sellable kernels ranged from 26.6% percent in LASER (an ornamental ex-PVP) to 100% percent in multiple formerly commercial ex-PVPs, including LH235. Using 12,952 SNPs, a significant association with marker S5_163144081, was identified for the percent of sellable kernels. This marker lies within, Zm00001eb240940 (*prh40*), an annotated protein phosphatase gene and has an effect of -3.3 (percent sellable kernels). The allele conferring a negative effect for percent sellable kernels is not evenly represented across heterotic groups; Iodent minor allele frequency (MAF) = 0.0%, Non-Stiff Stalk MAF = 15.0%, Stiff Stalk MAF = 4.1%, Unrelated/Flint MAF = 23.7%. The negative effect allele occurs at a lower-than-expected frequency in the Stiff Stalk heterotic group (p-value of 0.03) which contains inbreds traditionally used as the seed parent in hybrid seed production while it is more common than expected in the Non-Stiff Stalk heterotic group (p-value of 0.02), traditionally used as the pollen parent. Our results support that useful genetic variation exists for percent sellable kernels and that developing inbreds improved for this trait is a reasonable goal within a breeding program.

Gene / Gene Models described: *prh40*; Zm00001eb240940
Funding acknowledgement: United States Department of Agriculture (USDA)

P241

Leveraging above and below ground phenotyping of a maize synthetic population containing teosinte alleles in nitrogen limited field conditions to identify nitrogen efficiency alleles

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Nitrogen inputs are a major driver of agricultural greenhouse gas emissions and other environmental impacts. One solution is to breed crops that are more efficient at using nitrogen while maintaining yield. Modern breeding approaches have been successful at integrating beneficial traits into commercial varieties but domestication and improvement has decreased genetic diversity, resulting in the loss of many potentially beneficial alleles. Recapturing extant diversity still present in maize's undomesticated progenitor teosinte (*Z. mays parviglumis*) could aid in further gains in yield and other important agronomic traits. 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. 430 double haploid lines from this population, crossed to a common parent (PHZ51), were grown over two growing seasons in nitrogen limited condition and 270 phenotypes were recorded. Traits included agronomically relevant traits like grain yield, biomass, and nitrogen content. Root architecture traits were measured using 3 different technologies: analysis of 2-dimensional images of excavated root crowns, 3-dimensional x-ray computed tomography scans of excavated root crowns and data gathered from mini-rhizotrons in the field during the growing season. Elemental compositions of seeds for 20 elements were quantified using an inductively coupled plasma mass spectrometry pipeline. Lines were sequenced and a genetic map was constructed using the Practical Haplotype Graph (PHG) framework. Previously identified QTL were identified using this approach giving evidence for the viability of using the PHG framework in a large association study. Genome wide association scans were performed for each trait and significant associations were identified within each trait category, providing a basis for improvement of the nitrogen efficiency of maize.

Funding acknowledgement: National Science Foundation (NSF), Subterranean Influences on Nitrogen and Carbon (SINC) Center

P242 @RameshKannaMat1

Linking known and previously uncharacterized genes to roles in maize metabolism and whole plant phenotype using paired transcriptomic and metabolic data from a field grown maize diversity panel

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Here we sought to identify genes involved in producing variation in maize metabolism and link these genes to non-metabolic trait outcomes. We assayed 26 metabolites using GC-MS (Gas Chromatography-Mass Spectrometry) using leaf samples collected from 796 diverse maize inbred lines grown under field conditions during a two-hour period on a single day that roughly moderately proceeded flowering for the majority of lines. Resampling Model Inclusion Probability Genome Wide Association Study (RMIP-GWAS) was conducted for metabolite traits using a set of approximately 26 million SNPs (Single Nucleotide Polymorphism). And Transcriptome-Wide Association Studies (TWAS) were conducted using RNA-seq data obtained from paired leaf samples collected from the same leaves at the same time. We focused on six metabolites significantly associated with genetic variants. A total of 51 and 39 candidate genes were identified via RMIP-GWAS and TWAS, respectively. A Random Forest feature importance-based approach identified 10 genes which overlapped with the set of candidates identified via TWAS. Further RMIP-GWAS on 42 non-metabolic traits, including agronomic, hyperspectral and photosynthetic traits, identified 7 loci associated with variation in both metabolic and non-metabolic traits. A number of genes identified are known to play important roles in plant metabolic networks including Zm00001eb254940, Zm00001eb002790, Zm00001eb035010 and Zm00001eb142730, which encode the enzymes shikimate kinase, glutathione S-transferase, lipoxygenase and trans aldolase. These results enable future studies to delve deeper into the roles of these metabolites and genes in plant growth and development processes.

Gene / Gene Models described: ; Zm00001eb254940, Zm00001eb002790, Zm00001eb035010 and Zm00001eb142730
Funding acknowledgement: ARPA-E grant (ID: AR0001367)

P243 @BacherHarel

Longitudinal modeling of stomatal conductance potentiates the optimization of water-use efficient maize

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Slow-activating anion channel 1 (*slac1*) is a key regulator in stomatal closure, thus impacting water use efficiency (WUE) and productivity. The stomatal response to daily atmospheric parameters shapes the stomatal conductance (g_{sw}) dynamics and determines WUE. A drawback of breeding for WUE by reducing g_{sw} to minimize water loss is that it can decrease productivity. Our objective is to enhance WUE in maize by utilizing *slac1* alleles that allow for the differential regulation of stomatal closure, enabling the selection of higher g_{sw} predominantly in the morning when vapor pressure deficit (VPD) is lower, thus improving WUE without compromising productivity. To evaluate the feasibility of generating extreme g_{sw} phenotypes that have optimal diurnal dynamics, we created a series of hybrids by separately crossing a *slac1-mu* null allele and its non-mutant *slac1* counterpart with eight inbred lines showing variation in haplotype diversity at *slac1*, along with differences in *slac1* expression levels. We measured the diurnal dynamics of g_{sw} on the eight hybrid pairs, comprising *slac1*/*slac1* and *slac1*/*slac1-mu* genotypes, totaling 16 hybrids, in a replicated field trial over an approximately 11 h period on multiple days during the 2023 field season. By fitting a Gaussian model to the daily measurement of g_{sw} for each hybrid, three parameters were extracted: peak value of g_{sw}, time of maximum g_{sw}, and rate of daily g_{sw} dynamics. On average, we observed that the Gaussian model reasonably captured the data distribution (R^2=0.65), and the three parameters demonstrated a moderate level of broad-sense heritability. Among the eight hybrid pairs, two *slac1*/*slac1* hybrids were found to be significantly different (P<0.10) from their *slac1*/*slac1-mu* counterparts in terms of both time of maximum g_{sw} and rate of daily g_{sw} dynamics. Furthermore, g_{sw} was primarily altered in the morning when VPD is lowest, emphasizing the potential for optimizing WUE in maize.

Gene / Gene Models described: *slac1*; GRMZM2G106921
Funding acknowledgement: National Science Foundation (NSF), CROPPS (the Center for Research on Programmable Plant Systems)

P244

Maize hybrids display negative heterosis for induced defense while maintaining disease resistance

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Maize hybrids are often more vigorous and higher-yielding than their inbred parents. Plant-microbe interactions have been shown to affect the magnitude of such heterosis. To investigate whether this may be due to differences in defense responses, we examined disease resistance and basal immunity in inbred and hybrid maize. In a field experiment measuring resistance to multiple pathogens, we found that heterosis for disease resistance was inconsistent and dependent on both the disease being assessed and on the parental genotypes, while heterosis for plant height was consistent across genotypes as expected. We then tested basal immunity by inoculating several sets of hybrid and inbred lines with microbial elicitors. Hybrids exhibited negative heterosis for basal immunity, meaning a reduction in response to elicitors compared to parental lines. Activation of basal immunity can be energetically costly and reduce the rate of photosynthesis. Based on our results, we hypothesize that, compared to inbred lines, hybrid maize may be able to better regulate the induced defense response to minimize associated growth penalties while maintaining effective disease resistance levels.

Funding acknowledgement: National Science Foundation (NSF)

P245

Managing maize lethal necrosis disease using traditional and molecular breeding

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Maize lethal necrosis (MLN), caused by co-infection by maize chlorotic mottle virus (MCMV) and one of several potyviruses, is one of the most devastating virus diseases of corn. First identified in Nebraska, USA in the 1970s it has since been discovered in Southeast Asia, East Africa, and South America. Although major potyvirus resistance genes are well characterized in maize, most known MCMV resistance is highly quantitative and confers incomplete resistance. Thus, traditional and molecular breeding tools are desirable to develop varieties with elite MLN resistance. As part of a larger screening effort for MCMV resistance, a genome wide association study was performed using the Maize 282 Association Panel which led to the identification of variants on chromosomes 3, 4, 5, and 8 that are significantly associated with resistance. Notably, two of these variants co-localized with MCMV resistance quantitative trait loci previously identified in the dent corn inbred N211, further delineating these loci as potential targets for molecular breeding efforts. Five of the most MLN resistant lines discovered as part of our screening efforts were used to develop the maize synthetic population, OhMCMV-1, by recurrent selection for MLN resistance. The mean MLN disease severity was more than halved based on a single selection cycle and approximately 20% of the OhMCMV-1 S1 lines evaluated were completely asymptomatic to infection by MLN. The resources generated from these studies are invaluable for breeders interested in developing corn varieties with improved MLN resistance.

Funding acknowledgement: United States Department of Agriculture (USDA)

P246

Mapping freezing tolerance in tripsacum by bulk segregant analysis

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Increased variability in weather patterns is one consequence of climate change that may be addressable through changing agronomic approaches. These changes could include shifting planting zones or planting earlier to avoid excessive heat during flowering. However, these solutions result in an increased likelihood of crops encountering freezing events. To make these approaches viable in maize (*Zea mays*), it is imperative to improve freezing/chilling tolerance in maize. *Tripsacum dactyloides*, a close relative of maize that diverged roughly 650 thousand years ago, can be found across the Americas. As a perennial, this species must withstand elongated freezing temperatures when it occurs in northern latitudes. The genetic similarity between *Tripsacum* and maize may allow for freezing tolerance to be introgressed or genetically engineered into maize. We generated extensive genomic resources including newly generated Pac-Bio Hifi genomes for *Tripsacum dactyloides* as well as a mapping population constructed from crosses between plants sourced from northern and southern locations. To identify causal freezing tolerance genes, F_2 seedlings from an open pollinated *Tripsacum* F_1 population were subjected to -7°C overnight temperatures, and extreme phenotypes were collected as freezing tolerant and freezing susceptible bulks for bulk segregant analysis (BSA). Given the open pollination nature of the mapping population, we first estimated the paternal fraction in each F_2 bulk by regressing the F_1 genotypes onto F_2 bulk allele frequencies using genome-wide SNPs. With the identified parents, we then calculated the frequencies of the contributing F_1 gametes in windows, which allowed for accurate site-by-site allele frequency imputation and contrasts between susceptible and tolerant bulks. Preliminary results have shown once paternal contribution is accounted for and sites are imputed genomewide, noise is greatly reduced and candidate loci associated with freezing tolerance can be identified at higher resolution. In conjunction with further expression experiments, these QTL will inform future transgenic tests.

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

P247

Mapping tissue-specific disease resistance to *Xanthomonas vasicola* pv. *vasculorum* in maize

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The vascular xylem and nonvascular parenchyma tissues represent distinct habitats within a plant for pathogenic bacteria to colonize. Host plants often utilize different mechanisms to defend themselves against vascular and nonvascular pathogens. *Xanthomonas vasicola* pv. *vasculorum* (*Xvv*) is an emerging bacterial pathogen of maize that is threatening yields. It is described as a nonvascular foliar pathogen in maize, but a vascular pathogen in sugarcane. The maize *Xvv* pathosystem offers a unique opportunity to study how host resistance differs in response to the vascular and nonvascular lifestyles 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) vascular colonization by *Xvv* in maize is possible in susceptible maize genotypes; (ii) different inoculation techniques can be used to induce vascular or nonvascular colonization; (iii) resistance to vascular and nonvascular *Xvv* colonization in a recombinant inbred line population is independently controlled; (iv) there are striking differences in expression of genes relating to motility and virulence in *Xvv* when it is inhabiting the xylem versus the apoplast; and (v) there are significant expression differences for genes relating to plant defense in resistant and susceptible plants within the QTL intervals that we mapped. This research is significant because it is the first report of vascular disease induced by *Xvv* in maize, contributes to the limited knowledge regarding the genetics of resistance to *Xvv* in maize, and offers insights into how *Xvv* adapts to vascular and nonvascular lifestyles.

Funding acknowledgement: National Science Foundation (NSF)

P248

Multi-environment multivariate genome wide association studies identify candidate loci underlying cuticular wax accumulation and composition on maize silks.

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As global temperatures rise, precautions must be taken to mitigate the adverse effects of drought on water retention rates in crops. The cuticle is a hydrophobic layer that coats all aerial plant tissues. It consists of a matrix of cutin that is coated by and intercalated with very long chain cuticular waxes to form a barrier that limits rates of non-stomatal water loss and protects against other environmental stresses. We recorded the concentrations of forty-seven very long chain cuticular wax metabolites (hydrocarbons, fatty acids, fatty alcohols) from the silks of 448 inbreds of the Wisconsin Diversity panel, each grown in replicate across three year-by-location environments (Iowa and Minnesota, 2016-2017). Total accumulation varied 17-fold, with hydrocarbon, fatty acid, and fatty alcohol constituents displaying 18-, 23-, and 99-fold variation, respectively. To better understand the genetic mechanisms underlying cuticular wax variation, genome wide association studies (GWAS) were conducted for each trait individually. Further, we conducted multivariate GWAS using multiple wax traits or traits from multiple environments to uncover loci responsible for pleiotropic control of the cuticular wax profile. Combined, GWAS and multivariate GWAS yielded two-hundred unique, high-quality candidates, accounting for a high level of genetic variance across all measured traits; among these candidates are known stress responsive transcription factors and hormone receptors, as well as enzymes involved in wax biosynthesis. These candidate genes could be implemented directly into breeding programs or targeted engineering to ameliorate drought stress in silks, thereby improving pollen reception of the silks.

Funding acknowledgement: National Science Foundation (NSF)

P249

Multi-environmental RNA-seq reveals the extent of genotype-by-environment interactions as it relates to gene expression.

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Genotype by environment interaction (GxE) is a well known source of phenotypic variation, but there is more to be understood about the degree to which GxE impacts gene expression. Maize data were collected by the Genomes to Fields project resulting in a unique 3' RNA sequencing dataset which includes 576 samples at 5 locations with 27 hybrids. Using the RNA sequencing data, we quantified gene expression and created statistical models to partition genetic variation, environmental variation, and GxE variation for each gene. Models estimate the median of expression variation for genotype, environment, and GxE to be 10.3%, 23.8%, and 6.2%, respectively. We found 289, 1327, and 1 genes where over 50% of variation in expression is explained by genotype, environment, and GxE, respectively. These results show that for most genes, the environment has the greatest impact on gene expression, followed by genotype, then GxE; however, this level of impact on expression changes depending on the gene, where some genes' expression is mostly explained by GxE or genotype. We are continuing to evaluate the statistical power and suitability of this dataset for testing hypotheses related to GxE and will use our findings to inform the design of future experiments.

Funding acknowledgement: United States Department of Agriculture (USDA), Genome to Fields Initiative

P250

Optimizing a high-throughput screening method to identify the genetic regulators of VPD transpirational breakpoints in *Zea mays*

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Atmospheric temperatures are increasing, and extreme precipitation patterns lead to periods of severe drought. Both increased temperatures and drier growing conditions result in a higher atmospheric vapor pressure deficit (VPD). The VPD condition is calculated as the difference between the current level of moisture in the air and the potential saturation point. As VPD increases, the atmospheric water demand results 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 have observed transpirational breakpoints in *Zea mays* inbred and hybrid lines. However, in a *slac1-mu* stomatal mutant, which is unable to close its stomata, we did not observe a breakpoint. This result identified stomatal dynamics as the primary driver of VPD breakpoints in *Z. mays*. Variation in when and if a VPD breakpoint occurs has been observed in *Z. mays*, although the genetic mechanisms controlling VPD breakpoints are still unknown. Current methods of measuring transpirational breakpoints are time-consuming, limiting the number of genotypes that can be measured and our ability to identify regulatory genes. We have developed a new high-throughput screening protocol for measuring VPD breakpoints across a diversity of *Z. mays* lines. Currently, 28 *Z. mays* lines, including the NAM founders, have been screened for VPD breakpoints. We have found variation in the occurrence of VPD breakpoints and the severity of VPD that is required to provoke a stomatal response. Here we present our findings from our new breakpoint protocol and identify our next steps. Improving our ability to measure VPD breakpoints and identify genetic mechanisms regulating VPD response will provide new insights into stomatal dynamics and the optimization of water use efficiency in *Z. mays*.

Funding acknowledgement: National Science Foundation (NSF)

P251 @burnsmj7

Optimizing simulations for digital breeding that accurately parallels empirical data

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Computational and data-driven approaches to accelerate and optimize breeding programs are becoming a common practice among plant breeders. Simulations allow breeders to evaluate hypotheses and changes in a breeding scheme time- and cost-efficiently, but accurately simulating traits that match empirical data remains a challenge. Here, we address this problem by incorporating information about the genetic architecture (i.e., numbers of causative variants, effect sizes, and modes of gene action) from genome-wide association studies (GWAS) of common maize agronomic traits into simulations. Using at least 200 top GWAS hits as causative variants resulted in a high correlation of 0.54 between simulated and empirical trait data. In addition, reducing the original marker effect sizes estimated by GWAS models resulted in a more realistic partitioning of phenotypic variances from genotype, environment, and their interaction. Our results suggest that incorporating information from GWAS beyond statistical significance thresholds is valuable for simulating more realistic digital breeding phenotypes. To assess the utility of the simulated phenotypic data for digital breeding we are currently comparing the results of genomic prediction and machine learning models trained using the simulated data to the output of models trained on empirical data.

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

P252

Pangenome-based haplotype genomic prediction outperforms SNP-based prediction methods for North American corn breeding

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Genomic prediction has played a critical role in transitioning breeding programs to data-driven predictive and prescriptive breeding methods. Modern breeding programs routinely use genomic prediction with SNP genotypic data based on a single reference genome. Recently, teams in multiple cropping systems have demonstrated that pangenome haplotype databases can use low-pass skim sequencing data to impute genome-wide haplotype sequences for new individuals. Imputed haplotypes provide a more complete representation of the genome and more allelic diversity than do SNP genotypes, and we hypothesized that this improved representation of the genome would translate to increased genomic prediction accuracies. In this project we demonstrate that haplotype-based GBLUP models outperform equivalent SNP models in a large-scale North American commercial breeding program, with 3-5% improvement in prediction accuracy for yield. Preliminary results suggest that 60-70% of the gain in prediction accuracy is a direct result of denser genome coverage, with the remaining 30-40% gain coming from increased allelic diversity across loci. Haplotype-based genomic prediction provides an exciting addition to the breeder's toolbox that translates to improved genomic prediction models and increased genetic gain at scale.

P253

Performance of triple cross hybrids with varying genetic distances among inbred parents

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This study explores the potential of triple cross hybrids for organic seed production. Most commercial maize varieties are single cross hybrids. It is difficult to produce seed on single cross hybrids in certified organic fields because seed is produced on inbred parent lines that are small and easily overcome by the weeds that are often present in organic fields. However, triple cross varieties may be good alternatives, particularly from a seed production perspective. Unlike single cross hybrids, triple cross hybrids have three parents and seed is produced on a hybrid parent plant, rather than an inbred. With three parents, selection of parents for a triple cross hybrid is a challenge, especially with regard to heterotic groups. It is not clear how best to take advantage of existing heterotic groups to maximize performance. Moreover, the intricate interplay of genetic material from two distinct crosses in triple cross hybrids leads to a more complex genetic landscape compared to single crosses. Assessing genetic distances among parent lines may be useful for understanding the intricate relationship between parental lines and the resulting hybrid performance. To conduct a detailed genetic analysis, we genotyped a set of parent lines with 8K SNPs. The performance of a set of triple cross hybrids made with these parents was evaluated in multilocation field trials. Examination of the relationship of genetic distance between the parents and hybrid performance may lead to a deeper understanding of the complex genetic dynamics shaping the performance and adaptability of triple cross hybrids.

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

P254 @ThomasHartwig20

Phenotypic heritability in maize can be largely explained by genetic variation at transcription factor binding sites

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Comprehensive maps of functional variation at transcription factor (TF) binding sites (*cis*-elements) are crucial for elucidating how genotype shapes phenotype. Here we present the construction of a first-generation pan-cistrome of the maize leaf under well-watered and drought conditions. We quantified haplotype-specific TF footprints across a pan-genome of 25 maize hybrids and mapped over two-hundred thousand genetic variants (termed binding-QTL) linked to *cis*-element occupancy. Three lines of evidence support the functional significance of binding-QTL: i) they coincide with numerous known causative loci that regulate traits, including novel alleles of *Vegetative to generative transition1*, *Trehalase1*, and the MITE transposon near *ZmNAC111* under drought; ii) their footprint bias is also mirrored between inbred parents and by ChIP-seq; iii) partitioning genetic variation across genomic regions demonstrates that binding-QTL capture the majority of heritable trait variation across ~70% of 143 phenotypes in about 0.01% of the maize genome. Our study provides a promising approach to make previously hidden *cis*-variation more accessible for genetic studies and multi-target engineering of complex traits.

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P255

Phenotypic plasticity in a maize hybrid population across a precipitation and elevation gradient spanning Iowa and Nebraska

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As sustainability concerns regarding nitrogen fertilizer use, aquifer depletion, and the frequency of extreme droughts and precipitation events rise due to climate change, an improved understanding of phenotypic plasticity in crops has the potential to play an important role in improving crop resilience in the face of increasingly extreme and unpredictable weather and reduced access to agricultural inputs like nitrogen fertilizer and water for irrigation. Developing a better understanding of plasticity requires data from the same varieties in more environments than most individual research programs are able to generate. One notable long-running multi-environment field trial is the Genomes to Fields project, however; this project records only a small number of phenotypes, and incorporates only a small number of consistently represented hybrids across all locations. To generate an improved understanding of the role different component traits play in determining overall plasticity for grain yield, we generated data from a panel of 84 maize hybrids grown in replicated trials in 22 environments spanning a 700-mile range in the heart of the U.S. Corn Belt from the Iowa/Illinois border to western Nebraska. Environment played a large role in explaining variation in nearly all of the 23 phenotypes studied, but the relative contributions of genetics and nitrogen management varied among phenotypes, as did the contributions of genotype by nitrogen management and genotype by environment interactions. While individual maize hybrids varied in their average performance and degree of plasticity, no consistent major tradeoff between plasticity and good performance was observed in this study, which contrasts classical studies of phenotypic plasticity in a barley diversity panel studied by Finlay and Wilkinson. This may be explained by the high degree of selective breeding and improvement already applied to the elite public and private sector inbreds employed to generate the maize hybrids employed in this study.

Funding acknowledgement: National Science Foundation (NSF), United States Department of Agriculture (USDA), University of Nebraska-Lincoln Complex Biosystems Graduate Program

P256

Phenotypic plasticity in flowering time in maize-teosinte introgression populations shaped by interaction between domestication loci and diurnal temperature range

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Genetics, environmental factors, and their interaction shape the phenotype of living organisms. Flowering time is a critical trait of adaptation and domestication, exhibiting a diverse outcome of the gene-environment interplay. Understanding the underlying determinants of phenotypic plasticity, the property of a given genotype to produce different phenotypes in response to distinct environmental conditions, is a challenging topic in biology, evolution, and breeding. Herein, with eight B73-teosinte introgression populations evaluated for flowering time in 10 natural field conditions, the primary phenotypic plasticity pattern of flowering time was revealed. We found that diurnal temperature range from 9 to 29 days after planting during the growing season was the critical environmental index. Genetic loci, harboring domestication genes, including ZCN8, ZmCCT9, and ZmCCT10, were identified for reaction-norm parameters (intercept and slope) across environments and flowering time within individual environments through multi-allele QTL mapping. We observed the dynamic genetic effects of these loci along the environmental gradient and different parent-specific allele effects at individual loci. By exploring the genomic relationship and the environmental index, accurate prediction for flowering time was obtained with joint genomic regression analysis. The identification of genomic loci, environmental determinants, and their interplay enriches our understanding of genotype-by-environment interaction and how maize adapted to diverse climates.

Gene / Gene Models described: *ZCN8, ZmCCT9, ZmCCT10*; Zm00001eb353250, Zm00001eb391230, Zm00001eb418700
Funding acknowledgement: National Science Foundation (NSF), United States Department of Agriculture (USDA)

P257

Phenotypic selection of maize adapted to temperature extremes as a strategy to maintain productivity under global climate change

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One of the goals of the multidisciplinary, multi-institutional project called CERCA (***Circular Economy** that **Reimagines Corn Agriculture)*** is to create maize cultivars that can be planted several months earlier than the current practice, adapted to the cool 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 allow earlier planting and field emergence, including uniform germination under colder temperatures, winter dormant seeds, or deep planting to maintain the meristem below ground until spring. As part of the trait discovery team focusing on the seed germination life stage, we plan to evaluate diverse maize germplasm and related wild species under a range of cold soil conditions. To create the appropriate selection environment, we are using the soil-based LabField™ simulation table, an instrument that has been used to study seed germination behavior in a wide variety of crop species. We present here the results of our preliminary trials, in which we are experimenting with a range of parameters, most importantly soil temperature range, but also planting depth, trial duration, and planting density, by monitoring seed and seedling trait responses.

Funding acknowledgement: United States Department of Agriculture (USDA)

P258

Pipe dream or partitioning reality? Exploring extreme maize phenotypes and their role in programming senescence

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Carbon partitioning is a key regulator of maize senescence. In some genotypes the inhibition of seed set and subsequent buildup of carbohydrates in source tissue contributes to the phenomenon of non-pollination senescence. Alternative sink tissues can play a pivotal role in accepting excess photosynthate and delaying plant death. A preliminary screen of known non-pollination senescence sensitive and insensitive genotypes showed a strong correlation between cob density and staygreen traits, suggesting relevance of this structure as an alternative sink. Previous attempts to assess the importance of a cob-specific role have been confounded by non-pollination itself, which impedes cob developmental processes. To accurately test the sink strength of the cob, fully pollinated ears on isogenic lines with normal and high cob density are needed for comparison. Pipecorn is a specialty maize variety grown for its large, woody cobs to produce corn cob pipes. Pipecorn inbreds were used as high cob density donor parents and crossed to non-pollination senescence sensitive and insensitive recurrent parent lines B73 and PHG35. Among F_2 and BC_1S_1 populations, response to selection across backcrossing generations for both cob density and diameter traits has been notable. High density selection populations demonstrated transgressive segregation, suggesting synergistic genetic control of this trait compared to the recurrent parents. Pipecorn has undergone minimal genetic improvement, with all publicly available inbreds originating from the original pipe corn open-pollinated variety. New inbred lines are currently being derived from this open pollinated variety to develop pipecorn germplasm for future breeding applications and enhance its performance under modern agronomic conditions.

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

P259 @Harshita17_

Profiling population level gene expression from a field grown sorghum diversity panel for comparative analysis with maize

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Sorghum, unlike maize, is primarily self pollinated, creating fewer opportunities for recombination between dissimilar haplotypes. Linkage disequilibrium decays far slower in sorghum than in its close relative maize. As a result, genome wide association studies conducted in sorghum typically identify larger genomic intervals with greater numbers of candidate genes. Here we evaluate the use of transcriptome-wide association studies to identify candidate genes in sorghum, a method which has shown promise in soybean, another high linkage disequilibrium crop. Tissue samples were collected from 748 genotypes of SbDiv panel in a two hour interval during a field experiment conducted in 2021. RNA was extracted and RNA-Seq was used to estimate the expression levels of ~27,000 sorghum genes. TWAS conducted using measurements of flowering time and plant height for the same population identified 7 and 3 genes respectively. These included genes with known roles in the traits of interest in sorghum and others linked to the traits of interest based on data from homologs in maize, rice or arabidopsis. These initial results suggest TWAS can indeed provide accurate single gene resolution results in sorghum. Future work will integrate these results with GWAS, eQTL analysis and co-expression networks generated utilizing the same population as well as investigating changes in regulatory networks between maize and sorghum using a paired maize transcriptome dataset.

Gene / Gene Models described: *sbp29, sbp13, zap1, pebp14, mads76, sbp27*; Sobic.002G312300, Sobic.002G312200, Sobic.002G010100, Sobic.003G017200, Sobic.004G003434, Sobic.004G036900
Funding acknowledgement: National Science Foundation (NSF), United States Department of Agriculture (USDA), Department of Energy (DOE)

P260

Quantitative genetic analysis of variants contributing to variation in yield component traits in maize

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The genetic enhancement of corn yield is a centra/l aim in modern agriculture, crucial for advancing global food security and sustainable farming techniques. In this study, we utilized a dataset comprising of 750 corn genotypes from the Wisconsin Diversity Panel, collected over the years 2020 and 2021, to dissect the complex genetic determinants of yield. Being a composite trait, yield is determined by various factors including the number of plants per acre, ears per plant, kernels per row, and the weight of 100 kernels. We are calculating the Best Linear Unbiased Estimates (BLUEs) for these yield components to get the reliable phenotypic values for each analyzed trait. We are adjusting complex spatial variability present in field trials by considering genotype, environment, and genotype x environment interaction. (G, E, G x E). A resampling-based Genome-Wide Association Study (RMIP-GWAS) will be applied to identify genetic variants linked to the yield components using 1.6 million SNP markers. By examining the capacity of genetic markers to enhance yield prediction, we will hope for candidate genes expected to significantly influence yield variability. For potentially identified candidate genes mutants will be ordered and backcrossed to introduce these mutations into diverse genotypes. These new genotypes will be characterized to validate the effects of the identified genes on yield. Our research will aim to improve the prediction accuracy and breeding potential of yield for diverse populations in nearby future.

Funding acknowledgement: Department of Energy (DOE), Higher Education Commission of Pakistan (HEC)

P261

Reconsidering the genetic diversity underlying photoperiod-sensitivity for maize adaption to climate change.

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Flowering time, which is fundamental to local adaptation and crop productivity, is controlled by integrated networks of external and endogenous signals. This includes modules for sensing photoperiod and temperature. The spread of maize from its tropical origins to latitudes of the north temperate zone has shifted the control of flowering time toward temperature regulation and away from photoperiod sensitivity. Considering that rising temperatures will hasten flowering times in temperate maize, we hypothesize that reintroducing partial photoperiod-sensitivity can facilitate adaption to climate change. To better understand and model this reintroduction, the ecophysiological basis of flowering time and its genetic control must be elucidated. We test whether a field network constituting a photoperiod gradient can be used to define the physiological reaction norm for flowering time (PRN-FT) in maize, which decomposes autonomous and environment-dependent components of flowering time regulation in a genotype-specific manner. Our results show how selection restructures flowering time plasticity and has shaped the phenotypic space for flowering time in maize. Additional work is ongoing to: (i) characterize the PRFN-FT in near-isogenic lines with introgressions spanning photoperiod sensitive loci (tiling path of haplotypes from tropical lines introgressed into temperate genetic backgrounds), thereby testing the impact of reintroduced photoperiod alleles; (ii) use crop modeling to broadly evaluate the potential for using photoperiod sensitivity to counteract negative effects of warming on flowering time adaptation. Overall, this research provides a deeper understanding of the environmental and genetic controls of flowering time in maize, and helps evaluating the merits of reintroducing photoperiod-sensitivity in temperate-adapted maize varieties.

Funding acknowledgement: Agence Nationale de la recherche (ANR)

P262

Response of mature maize kernels to inoculation with *Aspergillus flavus*

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Crop growth and its interaction with microorganisms change yearly, mainly depending on local weather, making the agriculture particularly exposed to climate change. In the last seasons in Italy the climate was characterized by high temperatures that increased the parasitic ability of the fungus *Aspergillus flavus* and its power to colonize maize kernels, resulting in an increase in aflatoxins level. These molecules have the highest acute and chronic toxicity of all mycotoxins; hence, the maximal concentration in agricultural food and feed products and their commodities is regulated worldwide. An important way to contrast the fungus and high concentration of aflatoxins in maize kernel includes planting maize varieties that are resistant to *A. flavus*. In this study we suggest a simple methodology to test the susceptibility to *A. flavus* on candidate maize varieties before their release on the market.A panel of 100 breeding varieties of our reference collection was analyzed. Disease phenotypes were scored on artificially inoculated kernels using rolled towel assay. We confirmed the correlation between fungal infection with the reduced development of the seedling and recorded a rather wide variability in the response to fungal invasion between the genotypes analyzed, outlining four classes of susceptibility. The effect of the inoculation of *A. flavus* on genotypes marketed in different years (2001 *vs* 2010) suggests an increase of resistance in the latter year due to a success of plant breeding programs. We also investigated the combining ability effects on susceptibility to *A. flavus* to identify potential parents for hybrid development. The data collected with the laboratory screening will be used to perform a genome wide association study to unravel possible new genes involved in plant-pathogen interaction.

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P263

Response to arbuscular mycorrhizal fungi in field grown maize

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Arbuscular mycorrhizal fungi (AMF) are ubiquitous in cultivated soils, forming symbiotic relationships with the roots of major crop species. Studies in controlled conditions have demonstrated the potential of the symbiosis to enhance the growth of host plants. It is difficult, however, to estimate the actual benefit in the field, not least because of the lack of suitable AMF-free controls. Here, we report the use of maize genetic mutants to generate AMF incompatible sentinel plants that can be used as a baseline against which to evaluate the impact of the symbiosis. To estimate the overall (main) effect of AMF and characterize the impact of host genotype on symbiotic outcome, we have selectively incorporated AMF-incompatibility into genetic mapping populations. We are using these populations for trait mapping, estimating AMF, host genotype and host genotype x AMF effects by comparison of mycorrhizal and non-mycorrhizal families. We present evidence of a plant genetic trade-off between performance with and without AMF, indicating the importance of tailoring crop varieties to the AMF "environment", in the context of different types of agroecosystem. The approaches we present are applicable to other crop species, permit further mechanistic study and are scalable to larger yield trials.

Gene / Gene Models described: *Castor, Pollux, CCaMK*; Zm00001eb210900, Zm00001eb146490, Zm00001eb200350
Funding acknowledgement: United States Department of Agriculture (USDA)

P264

Self-organizing maps for clustering tropical maize inbred lines cropped under low and high nitrogen inputs in Brazil

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The success of a maize breeding program is very associated with the knowledge of genetic diversity (GD) of parental lines. To access GD, new tools such as artificial neural network can provide better results than usual statical methods. Self-organizing maps (SOMs), an unsupervised neural network, can improve the visualization of clustering onto a two-dimension grid. Thus, our objective was to employ SOMs to access the GD in a panel of 182 tropical maize inbred lines evaluated under different N inputs. Over three Brazilian summer seasons, the panel lines was evaluated under two N inputs: high N (HN) with 200 kg ha^{-1}, and low N (LN) with 20 kg ha^{-1}. The lines were laid out in a 13 x 14 alpha lattice incomplete design with three replications. We evaluated 14 traits, including those related to plant architecture and grain yield. To estimate the variance components, mixed model approach considering pedigree information, was employed. Then, the best linear unbiased predictors were used to construct the SOMs. The combined analysis and SOMs were performed across seasons under each N inputs. Notably, we observed reductions up to 30% in mass of thousand grain, grain yield per plant and grain yield under LN. Variance components were statistically significant ($P<0.01$) by likelihood ratio test for all traits under both N conditions. Analyzing the SOMs, we can identify four groups for LN and HN. In both environments, the weights of numerical phenotypic components are relatively bigger in neurons form by grain yield related traits. Under HN, internode length below the ear is important for clustering the panel of tropical lines. Besides, under LN the discriminating traits between the clusters are days to silking, leaf area and weight of thousand kernels. We concluded that SOM has the power to separate the line in different clusters.

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P265

SeqSNP-based genic markers revealed the genetic structure and identified candidate genes for low soil nitrogen tolerance in tropical quality protein (QPM) maize inbred lines

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Maize production in Sub-Saharan Africa (SSA) is threatened by low soil nitrogen (low-N). The identification of quantitative trait loci (QTLs) for low-N tolerance related traits is critical to increase the breeding efficiency through the integration of marker-assisted selection (MAS). In this study gene targeting markers (GTM) via SeqSNPs was used to determine the population structure and identify candidate genes for low-N tolerance. A total of 150 extra-early yellow, orange and white quality protein maize (QPM) inbred lines were evaluated under low-N and high-nitrogen (high-N). The inbred lines were genotyped using 2,500 SeqSNP markers which targeted genes previously reported for grain yield and other low-N tolerance related traits. Population structure analysis and phylogenetic tree both classified the genotypes into six sub-populations. Two, one, two and 10 significant SNPs were identified for low nitrogen base index (LNBI), low nitrogen tolerance index (LNTI), grain yield under low-N and grain yield under high-N respectively. The two SNPs for LNTI co-localized the putative gene hotspot GRMZM2G077863, belonging to the GDSL esterase/ lipase gene family and highly expressed in the roots of young seedlings at 6 days after planting and during tassel meiosis prior to flowering, which suggested a strong association of the gene with the traits. The putative genes GRMZM2G026137 and GRMZM2G004459 on chromosome (chr) 1, GRMZM2G111809 on chr 2, GRMZM2G380319 on chr 3, GRMZM2G442057 and GRMZM2G080314 on chr 6, GRMZM2G011213 and GRMZM2G090928 on chr 8, and GRMZM2G338056 and GRMZM2G150598 on chr 9 are involved in normal plant growth, tassel meiosis, root architecture, plant cell proliferation, cell growth, reproduction and post-embryonic development. The validation of these markers and the candidate genes in other population could make them useful for MAS to increase the selection efficiency and genetic gain in breeding for low-N tolerance.

Gene / Gene Models described: ; GRMZM2G077863, GRMZM2G026137, GRMZM2G004459, GRMZM2G111809, GRMZM2G380319,GRMZM2G442057, GRMZM2G080314, GRMZM2G011213,GRMZM2G090928,GRMZM2G338056,GRMZM2G150598

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P266 @snodgrasshopper

Single parent expression and heterosis in a diverse diallel F1 population

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The genetic mechanism underlying heterosis has been studied for over 100 years, resulting in several possible genetic models. The complementation model has the most empirical support, stating the masking of recessive, deleterious alleles underlies heterosis. This implies more distantly related populations that are distinguished by more genetic variants will produce hybrids with higher heterosis. An extreme pattern of differential gene expression called single parent expression (SPE) supports this hypothesis. SPE occurs when the hybrid and one parent express a gene while the other parent does not, leading to a greater number of genes expressed in the hybrid than either parent. Previous work has identified the patterns and genetic mechanisms of SPE in temperate maize, but wider crosses (*e.g.,* between temperate and tropical lines) have not been studied. The phenotypic consequences of SPE have also recieved little attention. Here, we developed a full diallel population from thirteen diverse inbred maize lines, including temperate and tropical germplasm, to address these questions. In 2020, fourteen traits related to plant architecture, yield, and growth rate were measured in Ames, IA, with most traits showing extreme heterosis. Greater heterosis was observed with more distantly related crosses and was not constrained to crosses between historically important heterotic groups. RNA-sequence data generated for each genotype in the half-diallel identified SPE. Across genotypes, the number of SPE genes ranged from 28 to 374, with significantly more paternal expressed SPE occurring and most SPE private to one or two genotypes. While the amount of SPE did not correlate to genetic distance of the parents (Pearson's correlation = 0.058), crosses between the same heterotic groups may share more SPE. The results of this study will strengthen the claim of SPE gene expression as an extreme example of the complementation model of heterosis.

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P267

Spatial analysis of stomatal patterning from computer vision-annotated images for GWAS/TWAS of a diverse biomass sorghum population

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Stomata serve an essential role in regulating the exchange of CO2 and water between leaves and the atmosphere. Stomatal conductance is determined by the combination of stomatal density (SD), stomatal size and stomatal aperture. Stomatal density is a function of a hierarchy of developmental processes acting along both the medial (i.e. midvein to margin) and longitudinal (i.e. ligule to leaf tip) axes of the leaf. In maize, proof-of-concept has been established for quantifying stomatal patterning as a kernel density distribution (KDD) that describes the self-repeating pattern of stomata on the epidermis. In doing so, it provides a means to break up a composite, complex trait such as SD into its subcomponents. This study aims to test the same approach in sorghum to perform a genome-wide association study (GWAS) and transcriptome-wide association study (TWAS) that can identify candidate genes underlying variation in stomatal patterning of a model C4 species. Optical tomography and AI-enabled computer vision were used to score stomatal positioning across 842 diverse accessions of photoperiod-sensitive, biomass sorghum. KDDs were generated for each genotype allowing for extraction of informative parameter-traits. Initial trait correlations provide evidence that there are some common trait-trait correlations shared between maize and sorghum particularly for cell size and shape while sorghum still shows some unique patterning attributes of its own such as wider distances between stomatal files compared to maize. On-going GWAS/TWAS analysis will identify potential gene targets for engineering altered stomatal patterning and water use efficiency in sorghum. In summary, this project provides evidence that the KDD method can be generalized across grass species allowing for both comparisons of overall morphology, and in the future could be used to explore the degree to which genetic drivers are shared between different grass model systems.

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P268

Target-oriented prioritization: a breeding decision tool for genomic selection

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Genomic data is a useful to predict trait values in the large breeding population before field trial, but the decision-making for select favorable individuals is challenging because the breeders often consider multiple traits. Personal experiences and field knowledge are valuable abilities for crop breeding. Thus, advancing "prediction to selection" remains to be important but unresolved. We propose an integrative multi-trait breeding strategy that incorporates trait predictions at the whole plant level to make a cohesive decision for selecting superior candidates via a machine learning algorithm, called Target-Oriented Prioritization (TOP). We found that the likelihood of identifying the valuable one from 300 breeding candidates was 0.3% for an untrained person, also as an impossible mission without breeding experiences. However, we will have the possibility of 60.8% for identification of the valuable one if empowered by TOP algorithm. It provided a potential breeding-decision tool for further moving forward to genomic breeding.

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P269

The core collection enriched for Korean maize (*Zea mays* L.) landraces reveals seed texture correlated morphological characters and novel multi-aleurone layer phenotypes

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The maize (*Zea mays* L.) is one of the important food crops and extensively studied for the crop genetics and breeding. In the Korean peninsula, it was introduced since the middle of the 16th century. Over 3,000 Korean maize landraces have been collected and deposited in the National Agrobiodiversity Center, South Korea. Most of the Korean maize accessions have been maintained as local landraces, implying preservation of diverse morphological traits with a wide range of variation. However, there has been a few studies for discovering the phenotypic characteristics of the Korean maize germplasm. To develop representative Korean maize landrace panel, we collected published phenotype data of 2,690 accessions. Total 12 categorical and 17 quantitative traits were evaluated to assess morphological diversity among collected accessions. Principal component analysis demonstrated that the variations of Korean maize germplasms were largely explained by traits including flowering time, kernel yield, and stalk diameter. In particular, the waxy and flint types showed distinct distributions by both stalk diameter and tillering patterns. We selected total 276 accessions as a core collection by Core Hunter 3 algorithm. Furthermore, we investigated not only the morphological traits in the field but also kernel phenotypes by observing dissected seeds with microscopy from the subset of the core collection. As a result, many novel germplasms with multiple aleurone layers were found, indicating that Korean landraces have maintained the original genetic variation that has been removed during the modern breeding program.

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P270

The dominance of gene expression controlled by *trans*-eQTLs contributes to heterosis of phenotypic traits in maize

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Heterosis, a pivotal genetic phenomenon, has been successfully harnessed in agricultural practices to enhance crop production yields. Despite extensive molecular investigations into the basis of heterosis, understanding its transcriptomic contributions has remained elusive. In this study, we present an integrative analysis encompassing seedling traits, adult agronomic traits, and molecular traits (transcriptomic data) derived from 200 maize inbreeds and their 600 F1 hybrids. Our findings reveal that the majority of hybrids (85.3%) exhibit a greater number of active genes compared to their inbred parents. We identify 7.8% of genes display positive mid-parent heterosis (MPH) for expression, while 7.9% show negative MPH. Notably, genes with both extremely low and high expression levels exhibit low heterosis, while intermediate-expressing genes demonstrate higher heterosis. Strong correlations between gene expression variation and expression MPH suggest that divergent selection on essential genes is more likely to result in heterosis. Heritability analysis indicates that heterosis traits, particularly for gene expression, are more heritable than traits *per se*. Results from expression Quantitative Trait Loci (eQTL) analysis highlight that the heterosis of molecular traits is predominantly regulated by *trans*-eQTL, with most MPH eQTLs exhibiting a complete–incomplete dominance pattern. Furthermore, we identify 452 eQTL hotspots governing the MPH of gene expression. Interestingly, target genes of three transcription factors under these eQTL hotspots are significantly enriched at ChIP-seq or DAP-seq peaks. In conclusion, our study underscores the significant contribution of *trans*-eQTL to gene expression heterosis, providing insights into the genetic basis of heterosis for agronomic traits at the gene expression level.

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P271

The maize ontogenetics: complexity, diversity and dynamics

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【Objective】 The formation of mature phenotype is related closely to the whole growth and development of organism, so investigating traits from the level of whole growth and development could improve the understanding of dynamic genetic basis of phenotype and expand more genetic resources from the time dimension. **【Method】** We monitored the whole growth status of the maize CUBIC panel using the high-throughput phenotyping platform. **【Result】** Over 1,002,240 RGB images were obtained, and 67 image-based traits (i-traits) from 18 time points were collected. We found the i-traits exhibited four diverse temporal patterns responsive to plant growth. The majority of the QTLs affecting i-traits are time-specific (84%), which largely explained the considerable portions of missing heritability of i-traits in the late growth phase. We simulated the accurate ontogenetic trajectories of maize genotypes, the diversity and genetic architecture of which were systematically understood. We found two genes that functioned differentially upon the plant growth, which greatly influenced the fine tuning of vegetative-reproductive transition in maize. **【Conclusion】** Our results provided the insights into the important traits from the ontogenetic perspectives, which proposed a new route for maize genome breeding via growth complementation.

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P272

The many contexts of genetic effects

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Technological developments have enabled larger-scale experiments capable of measuring multiple phenotypes throughout ontogeny in more diverse germplasm that has been grown in many environments. Although these data present many interesting statistical and computational problems due to their volume and diversity, quantitative genetic analysis of such datasets typically relies on the same fundamental framework: the one-locus model as used in, for example, QTL mapping. In this model, the genetic effects at a locus are estimated from the genotypic values. However, phenotypes develop over time (ontogeny), are influenced by the environment (plasticity), and result from inter-locus interactions (epistasis), among others. Each of these factors—singly or in combination—defines a different context for the estimation of genetic effects. While all of these factors interact in the expression of a phenotype, they are often studied in isolation to answer specific questions in biological sub-disciplines, using quantitative genetic analysis as a convenient analytical tool. Here, we use graphical and mathematical models to demonstrate parallels between the estimation of genetic effects in these different contexts and propose a unified framework to integrate discoveries from different disciplines and enable cross-disciplinary, combinatorial exploration of genetic effects in multiple contexts. Systematizing context-dependent genetic effects will help us better understand the landscape of genetic variation across diverse contexts by linking the results of gene cloning and molecular studies with statistical studies of phenotypic variation. This can be applied to improve the precision of selection decisions in plant breeding programs, inform complex trait dissection, and study the dynamics of phenotypic evolution in changing environments.

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P273

The recessive lesion mimic mutation (*les-2014*) results from the loss of function of the only *hpl1* gene in maize

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les-2014 is a recessive lesion mimic mutant that produces symptoms mimicking the grey leaf spot disease of maize. This mutant appeared in an EMS mutagenized population of Mo17, following a new mutagenesis approach that involves multiple rounds of mutagenesis over successive generations. We have named this approach NextGEM[2] (for next-generation mutagenesis version 2). *les-2014* was backcrossed to Mo17 over multiple generations, and an F2 population generated from cross of the mutant with B73 allowed the mutant to be mapped to the long arm of chromosome 4. Whole genome sequencing-based cloning was next used to identify the mutant gene underlying *les-2014*. Single nucleotide variants (SNVs) in the lower end of the long arm in chromosome 4 were filtered based on their allele frequency and nonsynonymous effects to determine one SNV unique to *les-2014*. The mutation was found to cause a missense mutation that changed the amino acid Ala to Pro at position 223 of the enzyme hydroperoxide lyase (HPL) that competes with the JA pathway enzyme allene oxide synthase to divert the oxylipin pathway towards green leaf volatiles (GLVs). That the correct gene was cloned was verified by showing that corresponding allele of a second *les-2014* mutant also sustained structural changes in the *hpl1* gene. Further characterization of the mutant and the *hpl1* gene is being done in collaboration with Mike Kolomiets of Texas A&M university. We are especially interested in knowing why a block in the production of GLVs ends up killing corn leaf cells.

Gene / Gene Models described: *hpl1*; Zm00001eb209760
Funding acknowledgement: National Science Foundation (NSF)

P274

The role of Grassy Tillers 1 in drought response of Sorghum bicolor

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Natural resilience to heat and drought has made *Sorghum bicolor* one of the most important cereal crops in the world. One of sorghum's unique abilities its unique ability to suppress the growth of tillers in response to abiotic stress. *Grassy tillers 1* (gt1) is one of the key genetic factors implicated in sorghum tillering. This research characterized the stages of tiller bud development and examined the role gt1 plays in the gene response networks for drought stress. Quantitative PCR (qPCR) was used to analyze the expression of gt1 in wild-type sorghum plants under drought and non-drought conditions. This data was paired with measurements of tiller bud length taken at set intervals after planting. Analysis of these measurements suggest that tillering plasticity in sorghum is impacted by water stress in the early stages of development but may only be visible in certain tissues due to the natural suppression of older tiller buds during normal development. RNA sequencing (RNA-seq) analysis was performed to assess the relation between gt1 and other known sorghum genes in response to water stress. CRISPR was utilized to create a gt1 knockout mutant line and a wild-type sibling line of sorghum plants. Tissue was collected once plants were visibly drought stressed. RNA-seq analysis revealed that, under water stress, several genes were upregulated along with gt1 in the wild-type sibling but not the gt1 mutant, suggesting they are part of a drought response network that involves gt1. This research shows that early tiller bud development is sensitive to drought and that response appears to be moderated by a gene network containing gt1. Understanding how drought affects tillering in sorghum is essential for optimizing the management of this crop in a rapidly changing climate.

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P275 @UMR_LEPSE

The shape of data: a new concept for understanding crop diversity

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We present topological data analysis (TDA) – methods for studying the shape of data in multivariate space – as an alternative to the long-standing statistical framework for studying crop diversity. This is demonstrated with multi-scale data combined from phenotyping platforms and field experiments on traits for crop growth and development. Using TDA to study genetic and phenotypic spaces in maize, we find that directed interrogation of topological structure provides unique insights into evolution and adaptation. At the phenotypic scale, TDA uncovers physiological divergence among genotypes with common flowering times, in addition to different evolutionary and breeding trajectories for phenotypic change. At the genetic scale, TDA also reveals how common flowering times arise from genetic heterogeneity of polygenic variation. For genetics and breeding, TDA helps to localize territories of multivariate space with taxa (populations, genotypes, or alleles) that are favorable for genetic dissection and environmental adaptation. By developing a biologically interpretable understanding of topological information, we define a new approach for generating testable hypotheses and actionable results for crop improvement.

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To explain and to predict: Genetic diversity, environmental context, and phenotypic plasticity

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Phenotypic plasticity is the property of a genotype to produce different phenotypes under different environmental conditions. Understanding the genetic and environmental factors behind phenotypic plasticity can help answer some longstanding biology questions and improve phenotype prediction. 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 the identified environmental index to quantitatively connect environments, a systematic genome-wide performance prediction framework 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 varied effects of genes, QTLs, and GWAS peaks along the environmental index visualized the environmental context of genetic effects, i.e., the gene-environment interplay. With additional multi-stage measurements of plant height, we profiled the genetic effect continua both along the environmental gradient and along the developmental stage. A conceptual model with three-dimensional reaction norms was proposed to showcase the interconnecting components underlying the phenotype: genotype, environment, and development, and at the three levels: single locus, multi-locus haplotype, and individual organism. 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 that further integration of development and physiology at the whole-plant level and gene expression network at the molecular level would enhance our understanding of mechanisms underlying phenotypic variation observed under diverse environments.

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Transformers for genomic selection

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Genomic selection is one of the ways maize breeding has sought to keep pace with global demand for agricultural goods. Conventional genomic selection strategies are regression-based and were originally designed to make predictions using moderate-to-small sets of genomic data. However, the data landscape has changed in the time since conventional methods were designed and the scale of genomic and phenomic data collection has dramatically increased over recent decades. Alternatives to conventional genomic prediction strategies will be required to better utilize big data and to close margins for prediction accuracy improvement that still exist in numerous traits and species. Artificial intelligence (AI)-driven models are poised to be the next evolution of genomic selection because of their suitability for big data and wide success at prediction in fields like natural language and medicine. However, many of the AI models developed for genomic selection have not yet consistently exceeded the performance of convention methods. But, AI models are rapidly evolving, and the current state-of-the-art AI model is the transformer which has not been extensively investigated for genomic selection. Transformers are a type of AI model that differs from others because of its use of attention and positional encoding. Attention evaluates which values in the input sequence are important for contextualizing a given input value. Positional encoding provides the order/positions of input values separately from the input values allowing for parallelization of sequential data. BERT (bidirectional encoding representations from transformers) is a type of transformer model that is suitable for this type of prediction task. Because of these capabilities, a BERT-based model pre-trained using Genomes to Fields (G2F) initiative maize data is being designed and investigated for genomic selection. Adapting existing AI methods to leverage domain knowledge in genetics and breeding may bridge the gap between AI and genomic selection.

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Unveiling the effects of organic nitrogen fertility and weed competition on corn hybrid agronomic performance and root architecture characteristics

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The domestication and improvement of maize (*Zea mays* ssp. *mays*) has focused on above-ground traits for crop improvement, neglecting below-ground phenes as selection criteria. Interactions between root architecture and nutrient capture affect plant growth and yield. The root system architecture influences the volume of soil exploration by the plant, whereas fine root traits affect water and nutrient uptake that ultimately influence yield. For the most part, root architectural traits have been overlooked in breeding programs because of challenges associated with phenotypic characterization and the lack of standardized screening methods. This study evaluated agronomic, and root architectural responses of maize hybrids grown with different levels of nitrogen inputs and under the manipulated weed competition in organically managed fields. The experiment applied an RCBD using a split-split plot arrangement, with three external nitrogen fertility inputs as whole plots (0, 112, and 224 kg/ha), ten hybrids as subplots, and two weed treatments (weed-minus, weed-plus) as sub-subplots. The study was conducted over three years (2018 to 2020) in four organically managed locations, with four replications per location. Hybrids were phenotyped for above-ground traits and assessed for below-ground root architectural traits using high-throughput image analysis. Significant variation was observed for above and below-ground traits across all treatment combinations. Higher nitrogen availability positively influenced above-ground traits (grain yield, plant height, ear height, and stem diameter). Increased nitrogen levels reduced root system complexity and increased root angles. Hybrids with simple roots and wider root angles obtained higher grain yield than hybrids with complex and narrow root angles. Hybrid performance varied significantly under weed competition. The study highlights the importance of considering below-ground phenotypes for crop improvement in maize and provides insights into the influence of nitrogen availability and weed competition on maize production in organically managed fields.

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Using remote and proximal sensing to quantify plant architectural variation across maize diversity

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Maize plant architecture is an often under-appreciated collection of traits that has been critical for its explosive yield improvements over the past century. Much of this improvement can be connected to simply planting more individuals within a given space, increasing from ~30,000 plants/acre to over 80,000 plants/acre with today's modern corn hybrids. The push for genotypes that can withstand tighter, more competitive growing space has resulted in the selection of hybrids with a more upright architecture. A maize plant with a so-called "ideal plant architecture" will have prone leaves near the bottom to efficiently absorb light while being less shaded by more upright leaves near the top. Since leaves and tassel branches are both lateral organs, some of the genes that control leaf architecture also play a pleiotropic role in the development of tassels. The interplay between these morphological traits, however, is not well understood. Based on our group's previously collected data on leaf angle and tassel branch number, we selected 426 accessions from the Ames population to maximize genetic as well as architectural diversity in these two traits. We planted this subset of lines in two fields at each of 2 locations (MO and IL) and used a combination of remote and proximal phenotyping platforms and sensors to collect data on whole plant morphology and physiology. Handheld phenotyping tools were used to measure spectral reflectance and absorbance as well as photosynthetic efficiency of leaves to correlate them with architectural traits. Additionally, UAVs, ground rovers and an imaging backpack was used to collect RGB images, multispectral and hyperspectral reflectance and LIDAR data. Algorithms are being developed to reconstruct plant architecture from the various images and point clouds and novel trait extraction for genome-wide association analyses. This will facilitate rapid phenotyping of traits of interest, e.g. leaf angle and tassel architecture, as well as more elusive traits that can't easily be measured such as how the architecture of individuals combine to form a canopy.

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Weighted records prediction for hybrid maize performance

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Modeling genotype-by-environment interaction (G×E) remains a challenging yet indispensable aspect of genomic prediction and breeding. Incorporating G×E into genomic prediction models significantly enhances prediction accuracy and guides breeding endeavors towards developing varieties with enhanced adaptability, stability, and performance across diverse environmental conditions, fostering sustainable and resilient agriculture. Within the Genomes to Fields (G2F) Initiative, the Maize GxE Project has meticulously evaluated over 180,000 field plots encompassing more than 4,500 maize hybrid varieties across 200+ environments in North America. Leveraging genotypic, environmental, and G×E information from this project, our study aimed to predict hybrid maize performance in different environments across the United States. In this pursuit, we introduced the Weighted Records Prediction (WRP) method for genomic prediction. WRP integrates weighted training records into the prediction model based on the precision of Best Linear Unbiased Estimates (BLUEs) and the similarity between the training and testing sets. Employing both linear mixed models and machine learning techniques, we assessed the prediction accuracy of this novel method. Our preliminary results showed WRP might improve the prediction accuracy for hybrid maize grain yield.

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P281 @justinscherer15

Analysis of de novo mutator transposon insertions across tissue types

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The Mutator (Mu) transposable element is a highly active DNA transposon that is native to Maize. Recently, targeted sequencing techniques have been utilized to identify the precise insertion locations of inherited Mu elements. This technique, known as Museq, has been used to quickly identify genetic knockouts. To better understand the de novo activity of Mu, we developed a modification to Museq, known as Museq2, to improve the quantifiability and sensitivity for detecting de novo insertions. Quantitative improvements in Museq2 were facilitated by the incorporation of molecular counting and suppression PCR to reduce the amount of non-transposon amplification. Museq2 has a detection threshold of 1 Mu insertion in 100,000 wild-type copies of DNA, allowing for precise analysis of de novo insertions over 5 orders of magnitude. We applied Museq2 to matched leaf, root, endosperm, and pollen tissue from individual plants and detected on average 80,000 de novo insertions per sample. Inherited and de novo insertions were distinguished by filtering insertions that were shared between pre-zygotically derived endosperm tissue and the zygotically derived tissue. De novo Mu insertions most frequently occurred in hotspots of ~1 kb, with thousands of specific hotspots identifiable across the Maize genome. We compared Mu insertion preferences across tissue types to evaluate the tissue specificity of Mu activity. Surprisingly, many hotspots were shared between tissue types. This finding may suggest that some of the biological determinants dictating Mu insertion preference may lack tissue specificity. Additionally, we find that Mu inserts into a palindromic target sequence motif just outside of Mu's target site duplication. Overall, these findings provide the first insights into the tissue-specific analysis of de novo insertions for any transposon and demonstrate that the sensitivity of Museq2 can be used to gain a great deal of insight into the well-studied Mutator transposon.

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Bi-directionally expressed enhancers harbor the molecular signatures of maize domestication

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Early maize lines were domesticated from Teosinte parviglumis, with subsequent introgressions from Teosinte mexicana. Domestication traits in modern maize include increased kernel row number, loss of the hard fruit case and dissociation from the cob upon maturity, as well as fewer tillers. Molecular approaches have identified several transcription factors involved in the development of these traits. However, these studies have also shown that a more complex regulatory network is responsible for these strong morphological differences than originally hypothesized, and our understanding of the tissue-specific regulation as well as of its variability across inbred lines is still lacking.In this study, we investigate the transcriptional regulation that resulted from the domestication process, focusing on conservation and variability across multiple tissues and inbred lines. We generated histone-modification and transcription factor ChIP-seq in parallel with transcriptomics datasets in up to 5 different tissues of 3 inbred lines which span the phenotypic diversity of maize inbreds, as well as the teosinte inbred TIL11. We then developed an automatized computational pipeline to integrate these datasets as well as publicly available data, and which could also be applied to any organism.
We identified regulatory regions in each tissue of each inbred, including those responsible for the tissue-specific expression of developmental genes. This integrative analysis refines the functional annotation of the genome, notably by identifying distal enhancers with bi-directionnally expressed enhancer RNAs, reminiscent of “super enhancers” in animal genomes. We show that, even though pollen grains are the most differentiated tissue on a transcriptomic level, and especially with respect to the regulation of transposable elements, ears show the least conservation both in gene expression and within regulatory regions, reflecting conspicuous morphological and physiological differences between maize and teosinte.The identification of the molecular signatures of the domestication process improves our understanding of transcriptional regulation in maize and can provide a framework for directed breeding strategies in plants.

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DNA glycosylase Mdr1 underlies a connection between DNA methylation and imprinted gene expression

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Maternal de-repression of R1 (Mdr1), a DNA glycosylase active in the central cell and in early endosperm development, removes methylation through a base excision repair pathway leading to asymmetric methylation of the maternal and paternal genomes. This asymmetric methylation has been hypothesized to lead to genomic imprinting of alleles, a phenomenon in which an allele is expressed in a parent of origin dependent manner . Previous research identified differentially methylated regions (DMRs) between mutant mdr1 and wild-type W22 endosperm, finding a weak relationship between Mdr1 DMRs and imprinting. While we can obtain single mutants of both Mdr1 and its paralog, Dng102, a double mutant is lethal indicating an essential function in gametophytes. Here, we analyzed the transcriptomes of wild type (WT) and mdr1 mutant endosperm 15 days after pollination to find 186 differentially expressed (DE) genes and TEs, of which 134 had a nearby or overlapping DMR. Helitrons are enriched among DE TEs as well as among TEs overlapping DE genes. Many (58%) of these features were previously identified as maternally expressed (MEGs) in a prior study of imprinting with an additional 19% (77.8% total) demonstrating maternally biased expression in WT endosperm, highlighting the role of Mdr1 in imprinted expression. However, of 240 previously identified MEGs, only 44% are DE in this dataset. We therefore aimed to understand the features distinguishing DE and non-DE MEGs. DE MEGs are enriched for endosperm-specific expression and DMR overlap. Interestingly, none of the MEGs with conserved imprinting in Arabidopsis were DE, so although Mdr1 alone can establish imprinting for many genes, there appears to be redundancy in imprinting control for critical genes. This research marks an advancement on the understanding of not only Mdr1's role in imprinted expression, but also the general relationships between demethylation and gene expression in the endosperm.

Gene / Gene Models described: *mdr1*; GRMZM2G422464
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Data mining dynamic transposable elements - Helitrons - using LSTM model

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While the mechanism by which *Helitrons* pick up host sequences is yet unknown, it is crucial to find out if *Helitrons* are capable of ensnaring fully functional genes and relocating them throughout the genome. Because lack typical transposon structural traits like target site duplications and terminal inverted repeats, they are difficult to detect computationally. We created an online search tool for identifying *Helitron* elements (http://bo.csam.montclair.edu/helitronscanner/). DNA sequences in FASTA format can be uploaded or copied by users. To make sure users feel comfortable with the well-designed GUI, four back-end functions have been developed. On the website, each function is in the upper right corner. For users who want to upload a file and receive a fast response from the server, the Quick-Run tool was developed. If necessary, the user can also specify the file's name, including the type of extension. For the purpose of data mining *Helitrons* in the genome of their interest, biologists will find this online tool to be extremely useful. For the time being, the 5' and 3' ends of *Helitron* elements are identified using the long short term memory (LSTM) concept. Our initial findings showed that training was successful on the training dataset and that performance improved on the test dataset.

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Dynamic genomic evolution via active LTR transposable elements in maize

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Long terminal repeats (LTR) retrotransposons, found across eukaryotes, are transposable elements which can copy and insert themselves into other loci within a genome. These transposable elements are similar to retroviruses in that they rely on reverse transcriptase to "copy and paste" themselves elsewhere in the host genome. From their own RNA transcript, they are able to use reverse transcriptase to make another DNA copy of themselves. This initially gave them the moniker, selfish genes. However, in the decades after the discovery of reverse transcriptase and LTR retrotransposons, it became known that non-genic DNA could have other functions. LTR retrotransposons are sources of mutation, inserting themselves into and mutating genes within their host organism. However, LTR retrotransposons are prone to mutation themselves, quickly becoming inactive, incapable of transposition. LTR retrotransposons constitute approximately 75% of the total genomic sequence of maize. The vast majority of these LTR retrotransposons are completely inactive. While these inactive retrotransposons can be useful for gleaning information about an organism's evolutionary history, the rare active LTR retrotransposons are of more interest. They are capable of causing mutations in current maize crosses and maize inbred lines. In this study, we created a tool for filtering out the inactive LTR retrotransposons and locating potentially active ones by adding extra functionalities to the freely available tool "LtrDetector" for locating LTRs. In an initial filter, LtrDetector found over three million potential LTR retrotransposons across thirty publicly available maize lines. After incorporating restrictions for size, an intact primer binding site, a polypurine tract, and coding sequences for reverse transcriptase, RNase and integrase, we narrowed down that large pool of candidates to just 27 potentially active LTR retrotransposons.

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Exploring the timing and regulation of the sporophyte-to-gametophyte transition (SGT)

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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 two facets of the SGT that determine the timing and scope of haploid selection: timing and regulation. 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, tobacco, and poplar. Alongside this, we are also studying genes that regulate the SGT in maize. Mdr1 and dng102 are DNA glycosylases that act redundantly during the SGT to activate a set of 58 pollen-specific genes, which account for >10% of the pollen transcriptome. Double mutants for mdr1 and dng102 cannot be transmitted through pollen, demonstrating that these two genes constitute a necessary component of genome regulation during the SGT. This research will fill fundamental knowledge gaps in the current understanding of the haploid generation of plants, especially regarding the timing and scope of haploid selection, which is relevant to breeding practices and agriculture.

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P287 @Citogenetica

Exploring tissue-specific variation in knob chromatin accessibility

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Maize knobs are heterochromatic domains distinguished by significant variations in their quantity, size, and distribution along chromosomes. At the DNA sequence level, these knobs are composed of tandem arrays featuring two distinct repeat types: K180 and TR-1. While the existence of specific knobs has been associated with adaptive traits, the exact molecular mechanisms responsible for these correlations remain elusive. Notwithstanding this understanding, the epigenetic state of knob regions still lacks comprehensive elucidation. To illuminate the dynamics of chromatin structure in K180 and TR-1 knob regions, we utilized chromatin accessibility data from four distinct tissues (ear shoot, endosperm, coleoptilar node, and root tip) of Zea mays B73v5. The preliminary examination of chromatin structure patterns entailed visual inspection utilizing differential nuclease sensitivity mapping data within a genome browser (genomaize.org). The findings revealed that TR-1 knobs consistently maintained a closed chromatin structure pattern. Conversely, K180 knobs exhibited tissue-specific variations, with the endosperm displaying the most closed chromatin, followed by the coleoptile node and ear shoot. Unexpectedly, the root tip demonstrated the highest levels of chromatin accessibility. To delve into the significance of this variation, a quantitative analysis employing a z-score-based approach was conducted. DNS-Seq data for each tissue were utilized for peak calling, and the positively identified peaks were subsequently overlapped with the two types of knob repeats. Z-scores, representing the deviations in standard units from the mean expected intersections, were computed. The results concurred with the qualitative analysis, depicting TR-1 with consistent heterochromatic patterns across tissues, while revealing notable variation in K180 knobs. Remarkably, the root tip exhibited an unexpected behavior, featuring a z-score nearly four times higher than expected for a heterochromatic region (-9.5). This implies that the chromatin of K180 knobs might undergo remodeling during maize development, challenging the conventional notion of static packing.

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P288 @ManishaMuna

Genetic determinants of hybrid decay in backcross populations of maize

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Hybrid incompatibilities triggered by wide crosses can limit plant breeders' ability to utilize broad allelic diversity present in wild relatives of crop species. An unusual type of hybrid incompatibility has been characterized between maize and its wild relative the Mexican teosinte - a "hybrid decay" in which the fitness of backcrosses progressively declined despite becoming practically isogenic to maize. Initial analyses suggest that this sickly phenotype may be caused by improper regulation of transposable elements (TEs) in hybrids. Here, we further explore both the phenotype and the genetic determinants underlying hybrid decay in backcross populations. We have confirmed the transmission of hybrid decay into two distinct genetic backgrounds, B73 and Mo17, and its persistence in 5 generations of backcrossing to the healthy parental line. Backcrossed individuals show reduced values for several traits, and there is a slight increase in severity of hybrid decay if it is transmitted maternally rather than paternally. We performed long-read whole genome sequencing using Nanopore on a single BC11 individual descending from the initial cross between W22 and the teosinte sourced from Valle de Bravo, Mexico that produces the hybrid decay phenotype. We are now characterizing structural variation between the BC11 line that exhibits hybrid decay and the W22 reference genome to determine the extent of unregulated TE variation in the genome.

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Genomic consequences of repeated chromosomal breakage

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When a chromosome has two centromeres a process known as the breakage-fusion-bridge cycle (BFB) generates reciprocal duplications and deletions in mitosis. BFB was first studied by Barbara McClintock and led to the discovery of transposable elements. The genomic effects of BFB are poorly understood due to the uncontrollable nature of the phenomenon. The development of an inducible centromere system addresses this issue by enabling BFB to be started and stopped. A transgene fusing the centromeric histone variant CENH3 to a DNA binding domain induces centromere formation over a large array of DNA binding motifs. This system will be used to induce BFB for a generation then generate progeny no longer undergoing BFB for genomic studies. This system has been successfully tested in preliminary work that yielded two transmissible BFB haplotypes. Transposon activity will be assessed prior to and following BFB to provide insight into the scale and locality of transposon activity. Changes in transposon activity have been linked to changes in epigenetic state which leads to the hypothesis that BFB is altering epigenetic regulation which activates TEs. Nucleotide and histone modifications will be surveyed prior to and following BFB to test this hypothesis. This research will revisit McClintock's original observations at the molecular level, aiming to understand the structural and epigenetic changes that lead to transposon activation.

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P290

High-resolution crossover maps highlighting the role of DNA methylation in meiotic recombination of maize.

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Meiotic recombination is a crucial process during which homologous chromosomes exchange genetic materials, impacting evolution and breeding. We investigated the influence of DNA methylation on meiotic recombination in maize *mop1* (*mediator of paramutation1*) mutants, which affect CHH (H = A, T, or C) methylation near transcriptionally active genes. We resequenced 97 and 110 backcrossed (BC1) individuals derived from female *mop1* mutants and female wild-types, and 122 and 94 BC1s derived from male *mop1* mutants and male wild-types, identifying crossovers (COs) at high resolution. Using a Hidden Markov Model, we identified 4,048 COs, with approximately 50% occurring within a 2kb interval in all populations. The *mop1* mutants display an increased frequency of COs at the distal ends of the chromosomes. Importantly, this effect is more pronounced during female meiosis as opposed to male meiosis. This observation indicates that the *mop1* mutation exerts a differential influence on the distribution of COs along chromosomes, with the impact varying based on the sex. Interestingly, the shift in the CO landscape in both sexes correlated with an enrichment of miniature inverted repeat DNA transposable elements. Whole genome bisulfite sequencing revealed that CO sites identified in *mop1* mutants had lower methylation levels than the same regions in WT, suggesting that *mop1* introduces new CO sites and alters the CO landscape by locally reducing CHH methylation. Furthermore, the study reveals that CO hotspots are more widespread in the *mop1* mutants, providing additional insights into the broader impact of the *mop1* mutation on the genomic distribution of CO. Moreover, our data showed that *mop1* mutants tend to introduce new crossover sites in the genomic regions with higher genetic diversity, the mechanism of which remains unclear. These findings shed light on the role of DNA methylation in meiotic recombination in maize at both local and global scales.

Gene / Gene Models described: *mop1*; Zm00001d003378
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Influence of the DNA glycosylase MDR1 on histone modifications in endosperm

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DNA demethylation by DNA glycosylases is essential for endosperm development. Demethylation of the maternal but not paternal genome results in genomic imprinting at genes where demethylation is required for transcriptional activation. In maize, two closely related DNA glycosylases, MDR1 (a.k.a. DNG101) and DNG102, redundantly perform this function, but MDR1 has a stronger effect. The majority of their target loci appear to be demethylated but not transcriptionally activated. To determine how transcriptional activation is related to chromatin structure, we used CUT&Tag in wild-type and mdr1 mutant endosperm with antibodies for two histone modifications, H3K56ac for active chromatin, and H3K27me3 for repressive chromatin. While many MDR1 target loci are associated with H3K56ac, more commonly they are associated with H3K27me3. Many of these loci have H3K27me3 specifically in endosperm, where they are demethylated. These results suggest that the removal of one form of transcriptional repression from the maternal genome (DNA methylation) is replaced by another (H3K27me3) to maintain repression, albeit by a different mechanism. We are currently searching for genetic features that could explain which loci gain H3K27me3 when they are demethylated by MDR1 and which loci gain H3K56ac. We also hope through this work to learn why some loci but not others are demethylated in endosperm, and how that demethylation affects chromatin structure and transcription. Understanding interactions between DNA demethylation and histone modifications will provide insights into the genes and processes that control endosperm development.

Funding acknowledgement: National Science Foundation (NSF)

P292 @BioJlynn

Maize AGO2 is involved in RNAi balance and epigenetic inheritance through the male germline

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RNA interference (RNAi) is a highly conserved mechanism that regulates gene expression at the transcriptional (TGS) and post-transcriptional levels (PTGS), with the outcomes of these silencing pathways differing profoundly in their persistence. Generally, PTGS represents a shorter-term silencing program while TGS, due to amplification of small RNAs (sRNA) from target loci, forms a feedback loop between sRNA and heterochromatin that is heritable through the germline. The silencing outcome of RNAi crucially relies on the interaction of sRNAs with Argonaute proteins (AGO), forming AGO-effector complexes that target homologous RNAs. While novel RNAi functions have evolved many times across eukaryotes, plants appear to have the most functionally diverse repertoire of RNAi machinery. The diversity of sRNA biogenesis factors coincides with an expansion of AGO genes, blurring the lines of how plants control the functional outcome of a given silencing sRNA. While seminal work in Arabidopsis has identified properties of sRNAs associated with AGO loading, the expanded AGO gene families in plants with large, repeat rich genomes like maize and rice highlights the need for a deeper understanding of the complex sorting mechanisms of plant AGOs. To address this need, we characterized maize AGO transposon insertion mutants and generated catalytic AGO mutants by targeting AGO PIWI domains with CRISPR-Cas9. We crossed these mutants into the *b1* paramutation system to investigate the role of maize AGOs in epigenetic inheritance. We found that maize AGO2 is involved in suppressing spontaneous silencing of *b1* and that loss of AGO2A affects the transmission of silencing through the male germline, leading to normal segregation of light and dark plants when crossed to *B-I*. These results suggest that AGO2A is involved in the establishment of *b1* paramutation and that AGO2A and/or AGO2B are involved in balancing TGS and PTGS, perhaps through competition with RdDM-clade AGOs for substrate sRNAs.

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P293

Maize domestication through the lens of transposable elements

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In maize, transposable element (TE) rich regions of the genome control most of the genetic variation for phenotypes. TEs are highly polymorphic and differ between individuals in presence-absence, sequence diversity, genomic location, and activity level. Investigating patterns of TE abundance and diversity have the potential to illuminate early maize domestication history, which has been difficult to research using SNPs or genic regions of the genome. In particular, specifics of where maize was domesticated are still unknown due to confounding population structure and continual gene flow between populations. Population-specific TEs can refine our understanding of the geography of domestication beyond the regional scale through identification of shared and unique TEs in maize and teosinte. Identification of teosinte population(s) with unique TEs present in all maize populations has the potential to indicate which populations of teosinte maize was domesticated from and in which geographical regions early maize domestication occurred. To test this hypothesis, we compared TE composition of 195 maize and teosinte individuals across 5 populations using short reads analyzed with RepeatExplorer2, an unsupervised clustering algorithm. We then compared overlap and divergence of TE clusters between populations of teosinte and maize while controlling for the age of the TE insertion and underlying population structure. Comparison of different TE clusters allows for detection of candidate geographic regions for early maize domestication. Updating our understanding of maize domestication can inform current research on maize diversity and evolution.

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P294 @ClaireCodes

TIPs and tricks for identifying transposable element insertion polymorphisms using short-read data

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Transposable element insertion polymorphisms (TIPs) are transposable element (TE) sequences not found in the same location between individuals. TIPs have been shown to contribute to dramatic differences in phenotype which has compelling implications for crop improvement and genome evolution. Historically, a major challenge with characterizing TIPs on a genome-wide level is access to high quality genome assemblies, precise annotation of reference transposable elements, and algorithms that can accurately use this information with short-read data. Now, with access to genome assemblies from 700+ plant species, and multiple assemblies within some species, we are poised to study polymorphic TE insertions at the genome and population levels. There are several tools available that use short-read resequencing data from the population together with reference TE annotations to identify novel TIPs. Here, we benchmark six programs using Arabidopsis and maize resequencing data, with regards to precision, sensitivity, F1, FDR, and power of discovery relative to a gold standard set of TIP calls from whole genome alignment of reference quality genome assemblies. One substantial drawback to these six programs is each program's runtime and memory efficiency within large complex genomes. Consequently, we developed a fast and memory efficient new tool, SWIF-TE (Short-read Whole-genome Insertion Finder for Transposable Elements), which is built to balance computational resources and accuracy for practical use in species with large genomes. We further characterize how SWIF-TE provides variable accuracy based on mappability of the region, genomic context (e.g. genic vs non-genic regions, centromeric regions, etc.), and TE classification. This benchmarking study provides valuable insight into the computational tools that are best suited for the identification of TIPs based on the biological questions of interest and the data and information that is available for the analysis.

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P295

The maize ufo1 and its mysterious function in kernel development

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The endosperm of maize (*Zea mays* L.) kernel is comprised of multiple specialized cell layers playing important roles in nutrient transport and storage. Here we characterized the gain and loss-of-function mutants of the maize *unstable factor for orange1* (*Zmufo1*) that had cell differentiation defects and elevated accumulation of reactive oxygen species, oxidative DNA damage, and cell death phenotypes in the basal endosperm regions of the developing kernels. Antioxidant supplementation during *in vitro* culture of developing kernels alleviated the excess ROS, reduced DNA damage, and restored cell morphology in the basal endosperm. The transcription of endosperm development, redox homeostasis, oxidative stress response, and chromatin remodeling-related genes were affected in *Zmufo1* mutants. Overexpression of *Zmufo1* also altered histone methylation marks in the enhancer and gene body regions of *pericarp color1* (*Zmp1*), the classical marker gene that led to the discovery of the *ZmUfo1-1* gain-of-function spontaneous allele by Charles Burnham in 1960. *Zmufo1* encodes a nuclear protein with very low similarity with known proteins that interact with diverse partners, including chromatin remodelers and transcriptional regulators. Collectively, the results indicate a role of *Zmufo1* in basal endosperm development through maintenance of redox-homeostasis, chromatin remodeling, and transcriptional regulation-dependent mechanism.

Gene / Gene Models described: *ufo1*; GRMZm2G053177
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P296

Vgt1: a *cis*-regulatory element regulating flowering time and growth speed

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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. Although thousands of candidate *cis*-regulatory sequences have been identified in maize, few *cis*-regulatory elements have been well characterized. We are studying the function of *Vgt1*, a predicted regulatory element located about 70 kb upstream of the floral repressor gene *ZmRap2.7*, in more detail. Consistent with a function as enhancer of *ZmRap2.7*, *Vgt1* contains an accessible chromatin region. It however lacks significant enrichment of H3K9ac, a histone modification often observed at active regulatory sequences. Silencing of *Vgt1* by RNA-directed DNA methylation results in earlier flowering, and in addition accelerates the growth speed. Expression analysis by RT-qPCR on different leaf areas during plant development indicated *ZmRap2.7*downregulation in leaf 4 of V3 plants upon silencing of *Vgt1*. Consistent with the role of *ZmRap2.7* in flowering, DNA methylation of *Vgt1* resulted in earlier and higher *ZCN8*, *MADS67* and *ZMM4* expression in leaves during plant development. RNA-seq analyses confirmed the downregulation of *ZmRap2.7* expression in *Vgt1* silenced plants. Altogether our data are consistent with *Vgt1* acting as enhancer of *ZmRap2.7*.
Weber et al. (2016) Trends in Plant Science 21, 974. doi: 10.1016/j.tplants.2016.07.013. Oka et al. (2017) Genome Biology 18:137. doi: 10.1186/s13059-017-1273-4. Schmitz, Grotewold, Stam (2022) The Plant Cell 34:718. doi.org/10.1093/plcell/koab281.

Gene / Gene Models described: *ZmRap2.7*; Zm00001eb355240
Funding acknowledgement: EU FP7 MSCA ITN EpiTRAITS

P297

mRNA splicing variation in *mop1-1* mutant maize

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In plants, RNA-directed DNA methylation (RdDM) is an epigenetic pathway involved in the establishment and maintenance of heterochromatin. One mutant genotype of maize called mop1-1, deficient in a crucial component of the RdDM pathway, has been used extensively to study the effects of RdDM on different cellular and genetic processes. In other species, chromatin structure has been shown to be associated with mRNA splicing, and some variability in splicing has been observed previously in mop1-1 mutants. It is hypothesized that mop1-1 mutant maize has higher overall splicing variability than wild type. To examine variations in splicing across these genotypes, RNA sequencing data from wild type and mop1-1 maize is being evaluated. Preliminary data using the isoform percentages calculated for specific genes of interest showed many had higher splicing variation in mop1-1 mutants than in wild type. To examine splicing variation in this genotype more broadly, RNA sequence data was run through a program called VaSP, an R package used for quantification of variations in splicing events in a population. This program generates a Single Splicing Strength (3S) score for each intron splicing event based on junction reads and gene-level reads. Preliminary data and current results of the analysis with VaSP will be presented.

Gene / Gene Models described: *mop1*; Zm00001eb080370

P298 @ilksen_tt

Crop domestication in *Zea mays* has contrasting effects on microbiome community structure in above and below-ground microbiota

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Contemporary crop cultivars are the products of selection during domestication, spread, and breeding processes. The focus of recent breeding efforts has been on maximizing yield, though yield is known to trade off with stress tolerance. Our understanding of crop microbiomes and how they may have been shaped by those processes is rudimentary despite their relevance to plant fitness. In natural settings, crop wild ancestors such as Balsas teosinte (*Zea mays parviglumis*), the ancestor of maize, rely in part on their microbiomes to tolerate a variety of environmental stresses. We investigated whether the known de-escalation of plant defenses in elite maize cultivars compared to wild and cultivated ancestors is linked to alterations in their microbial communities. We focused on the leaf endosphere and rhizosphere microbiotas of teosinte (*Zea* spp.) and maize accessions spanning the evolutionary transition from a perennial life history (*Zea diploperennis*) to annual wild (Balsas teosinte) and domesticated (maize landraces and elite inbreds) life histories. We found that the rhizosphere microbiota of landrace and elite maize cultivars exhibited greater microbial diversity and richness compared to teosintes, whereas we found the opposite in leaf endosphere. Notably, the leaf endosphere harbored more biomarker microbiota than the rhizosphere, and domestication strongly affected both leaf- and root-associated microbial communities. Furthermore, patterns typical of microbial dysbiosis in the leaf endosphere microbial community were coincident with domestication, consistent with the Anna Karenina principle. This is the first evidence of dysbiosis associated with crop domestication. Finally, we share preliminary findings on utilizing Balsas teosinte leaf endosphere microbes for developing biopesticides. Understanding the effects of maize domestication, spread, and breeding on the leaf endosphere and rhizosphere microbiota of commercial cultivars is crucial for harnessing teosinte beneficial microbes to develop biocontrol agents and biofertilizers to improve maize resilience to biotic and abiotic stress.

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P299

Determination of genetic and epigenetic regulations of meiotic recombination under domestication in maize

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Meiotic recombination involves the exchange of genetic material between homologous chromosomes, playing a key role in evolution and genetic diversity. Meiotic crossovers are not evenly distributed on chromosomes; instead, they are enriched in hotspots controlled by both genetic and epigenetic factors. In maize, meiotic recombination has significantly contributed to its domestication. However, the specific mechanisms of meiotic recombination influencing maize domestication remain largely unexplored. In this study, our objective is to elucidate the genetic and epigenetic mechanisms orchestrating meiotic recombination during the domestication of maize. Our investigation encompasses a comprehensive range of maize lines, including 5 teosintes, 7 landraces, and 16 cultivars. These lines were selected because they maximize genetic diversity and thus are most likely to show variation for recombination. Currently, we have obtained data from two teosinte lines (BT1 and BT2) and 14 cultivars. Our results reveal significant differences in genetic distance and crossover numbers between the two teosinte lines, indicating wide genetic diversity between them. Moreover, estimation of the recombination rate shows that BT2 recombines at a lower rate compared to BT1, further highlighting the divergence between these two teosinte lines. Comparatively, while one of the teosinte lines displays the highest number of crossovers, the other exhibits the lowest crossover numbers in comparison to the 14 cultivated lines, indicating substantial variations between these groups. Our next step is to complete the analysis of the remaining maize lines and perform further investigations to enhance our understanding of the mechanisms involved in meiotic recombination in maize.

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P300

Distribution and impact of functional *glossy15* haplotypes on phase change, flowering, and grain drydown

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Maize grain moisture dynamics are key determinants of yield, quality, and harvest timing. Genetic factors controlling the developmental transitions from juvenile to adult and vegetative to reproductive phases ultimately govern the onset of drydown. Among these genetic regulators is *glossy15* (*gl15*), best described for its role in the transition from juvenile to adult developmental phases. A panel of diverse maize inbreds was used to identify six functional *gl15* haplotypes (A-F), explaining much of the variation in juvenile phase transition among dent corn lines. The prolonged juvenile identity from *gl15* overexpression delayed the onset of flowering, impeded drydown, and reduced harvest index. To determine whether early phase transition might improve drydown dynamics, the *gl15-H* null allele was introgressed into a panel of eighteen North American Yellow Pearl Popcorns and developmental traits were phenotyped, including timing of phase transitions, drydown, and grain quality traits. When the haplotypes of these eighteen accessions were determined through Sanger sequencing, most accessions belonged to two of the six haplotypes identified in dent germplasm. However, a seventh haplotype was identified (G), seemingly unique to popcorn, that delayed juvenile transition and flowering. A broader investigation into the distribution of this haplotype in a popcorn diversity panel revealed that despite occurring in 44% of North American Yellow Pearl Popcorn landraces, no North American Yellow Pearl inbreds carry haplotype G. Similarly, investigation into Latin American Pointed Rice Popcorns revealed that although 71% of landraces possess this haplotype, only 14% of their inbreds carry it. Despite this haplotype appearing entirely absent in dent germplasm and seemingly unique to popcorn, it is present within teosinte, suggesting that popcorn alone maintains genetic diversity once present in the maize ancestor. The changes in haplotype G presence suggest that prolonged juvenility and delayed drydown have been selected against under modern breeding efforts.

Gene / Gene Models described: *gl15*; Zm00001eb387280
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P301

Evolutionary mining of cold tolerance alleles in maize for future farming sustainability

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Shifting 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 hypothesize 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 for 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 into maize crop improvement and global agricultural sustainability.

Funding acknowledgement: United States Department of Agriculture (USDA)

P302

Genetic legacies of the first ancient South American State in archaeological maize

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Moquegua Valley in South Peru has an ancient legacy of maize cultivation and colonization preceding European contact. 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. Moquegua Valley inhabitants gradually increased cultivation of maize over several thousand years, 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. By tracing highland and lowland alleles and studying trait selection over 1,500 years of human occupation, this research provides insight into the co-evolution of humans and maize. We document early maize traits under selection and explore the impacts of Tiwanaku colonization of the Moquegua Valley. Our methodology analyzes ancient DNA from AMS-dated archaeological maize specimens spanning thousands of years to understand how Tiwanaku state colonization influenced maize genomics, advancing our comprehension of the human-maize relationship. Comparisons to modern maize help assess the legacy of these processes within current germplasm. Moreover, broader impacts will empower of 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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P303

Genome-wide association studies to establish the genetic basis of agro-morphological and climatic factors in wild maize relatives

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Maize's close wild relatives are teosintes, which have a wide ecogeographic distribution in Latinamérica spanning extreme ranges of precipitation and temperatures. The identification of the genetic variants associated with adaptation to such conditions is a necessary step in order to better monitor, conserve and use such diversity in applied projects. Knowledge in the form of sequenced genomes, transcriptomes, and especially loci identified to underpin adaptations to extreme growing conditions, provides a valuable resource for future breeding and in situ conservation projects aimed at providing maize races adapted to the challenges caused by global climate change. As part of our research, our Mexican collaborators performed an extensive teosinte sampling, including approximately 4,000 individuals of 276 populations of all the 7 teosinte species and subspecies distributed in Mexico. These samples were phenotyped in a greenhouse common garden, and genotyping by sequencing (GBS) was applied generating about 30,000 SNPs. We analysed the genetic population structure spanning seven teosinte taxa, phenotypic and eco-geographic variability elucidating relationships between genotype, habitat climate, and phenotype. A genome-wide association study (GWAS) was also done to elucidate the genetic basis of various morphological and climatic traits including plant height, plant surface area, number of tillers, the weight of 100 kernels, relative humidity, precipitation, and solar radiation among others. This was achieved using the fixed and random model circulating probability unification model (FarmCPU) and Multiple Loci Mixed Model (MLMM). Our findings highlight significant genetic diversity within teosinte populations, underscoring their adaptive responses to diverse climatic conditions. We identified candidate genes located in or near SNPs associated with these traits. We observed that the genetic loci associated with climatic factors differ across teosinte populations, emphasising the importance of preserving the genetic diversity of all populations. Consequently, our research indicates substantial potential for maize breeding, leveraging the rich genetic diversity within teosinte populations and therefore the importance of preserving the genetic diversity of all populations.

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P304 @GrameneDatabase

Gramene 2024: A comparative resource on plant reference genomes, pan-genomes and pathways

(submitted by Nicholas Gladman <gladman@cshl.edu>)

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Gramene, established in 2002 (https://www.gramene.org), is a pivotal open-source plant genome database facilitating agricultural research. Focused on functional genomics in both crops and model plants, it boasts 150 plant reference genomes on the main site and 100+ crop-specific genomes on subsites (e.g., https://maize-pangenome.gramene.org with 37 maize genomes). This extensive resource enables maize researchers to seamlessly transition from genes in other species to their maize counterparts, evaluating expression, identifying variants, visualizing genomic context, and exploring metabolic pathways. Collaborations and adherence to FAIR principles have fueled Gramene's growth. Release 68 (December 2023) presents ~157K gene family trees, including ~2K maize-specific gene families, shedding light on eukaryotic evolution. The release incorporates tools for amino acid alignments, gene neighborhood conservation, and whole-genome DNA alignments, enhancing gene structure annotation. Genetic variants for 17 species, encompassing 50 million maize HapMap2 and Panzea 2.7 GBS SNPs, are available with detailed views and filters. Plant Reactome features curated rice pathways extended to 130 species, encompassing 270 projected maize pathways (B73 v4 and v5). Integration of EBI Atlas provides gene expression data for 26 species, including maize, offering insights across tissues and conditions. Recent enhancements include eFP Browsers for advanced gene expression visualizations and the CLIMtools portal for interactive views of environmental data and genome-wide associations in Arabidopsis and rice. The platform supports gene queries through text-based searches and sequence-based BLAST. Gramene's funding, from diverse sources including USDA-ARS, Wellcome Trust, BBSRC/NSF, and EMBL member states, underscores its sustainability. The integrated gene search interface now includes curated papers, enriching gene function understanding by linking well-annotated homologs with gene models from other species.

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P305 @SENTHILKUM72312

Maize x teosinte synergy: A holistic vision for climate-resilient crop evolution through integrated innovative breeding strategies

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Maize, a cornerstone cereal crop, has witnessed transformative changes through domestication, leading to the depletion of critical adaptive alleles. This genetic bottleneck presents challenges amidst climate change, necessitating the reintroduction of allelic diversity for improved grain yield and quality. This study pioneers innovative breeding strategies that seamlessly integrate conventional and advanced tools to address these challenges. Teosintes, the untamed kin of maize, harbour a reservoir of potential traits, including drought and excess soil moisture stress tolerance, disease resistance, and improved yield and quality characteristics. Focusing on specific teosinte species—*Zea mays* subsp. *parviglumis*, *Zea mays* subsp. *mexicana*, *Zea diploperennis*, and *Zea nicaraguensis*—our aim is to enrich maize germplasm through a holistic breeding approach. Crossbreeding *Zea mays* subsp. *parviglumis* with maize lines has revealed Quantitative Trait Loci (QTLs) responsible for diverse traits, including Banded Leaf Sheath Blight (BLSB), Maydis Leaf Blight (MLB), red flour beetle resistance, high protein content, and alterations in flowering behaviour. Striking characteristics such as multiple ears, a stay-green phenotype, and adaptability for high-density planting have been observed. Exploiting the waterlogging tolerance potential of *Zea nicaraguensis*, we create populations to map QTLs and genes, developing tolerant lines for integration into the maize breeding program to yield waterlogging-tolerant hybrids. Challenges arise from undesirable genes and alleles in wild teosintes, potentially hampering the efficiency of introgressed lines. To overcome this, an innovative strategy is employed, curbing linkage drag through random mating among backcrossed inbred lines. The integration of *in-vivo* doubled haploid (DH) technology further expedites allele development and fixation within a short time frame. This synergistic approach, harmonizing the forces of nature and technology, aims to accelerate the domestication of valuable wild alleles. The resultant fortified germplasm holds immense promise for developing climate-resilient maize hybrids, fostering sustainable production, boosting productivity, elevating farmer income, and creating new employment opportunities.

P306

Molecular evolution of a maize hybrid barrier over 12 million years suggests epistatic silencing

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Maize and its wild relatives teosinte can all readily hybridize, yet they remain distinct. Mating incompatibility loci provide one mechanism that can allow populations to maintain reproductive isolation. In Zea mays, three complex mating incompatibility loci encode genes that disrupt directional pollen tube growth down the silk. When a maternal plant receives pollen with incompatible alleles, fertilization is impeded, creating a prezygotic reproductive barrier. However, this barrier is not complete. Infrequent fertilization of incompatible gametes facilitates introgression of incompatibility genes into other populations. Previous modeling shows that each factor should only undergo brief periods of strong selection, on a timescale shorter than time to speciation in this clade. Against this expectation of transient benefit, we find evidence of syntenic gametophytic factor loci across modern maize lines, teosinte subspecies, other members of the Zea genus and Tripsacinae subtribe, and species as diverged as Sorghum bicolor. All of the loci display presence absence variation and copy number variation. To reconstruct the evolutionary history of these complex loci, we classify haplotype diversity at all three loci in over 30 Zea mays genomes, identify syntenic loci in related species, construct gene trees of known and candidate functional genes, and analyze rates of molecular evolution. We find evidence of potentially functional reproductive barrier loci and genes in lineages that have been estimated to be twelve million years diverged. Over millions of years, these genes have undergone selection and evolved to be distinct barriers. We find differences in predicted protein structures that reflect this distinction. We also find evidence of loci driving epigenetic silencing of interacting loci. These loci may have played a role in speciation in Zea.

Funding acknowledgement: National Science Foundation (NSF)

P307 @mcstitzer

Revisiting the subgenome ancestry of maize

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Early cytological, molecular, genetic, and genomic studies hypothesized maize originated from an allopolyploidy event, due to the presence of duplicated regions throughout the genome. This whole genome duplication occurred 8-13 million years ago, generating the ancestor of both *Zea* and *Tripsacum*. Maize has since undergone extensive biased fractionation as it rediploidized, resulting in ten chromosomes. A recent chromosomal-scale genome assembly of *Tripsacum* confirmed that its 18 chromosomes retain two largely intact syntenic subgenomes, but left questions as to what taxa contributed to the allopolyploidy event. Here, using over 500 newly sequenced species in the tribe Andropogoneae, we reconstruct genome-wide phylogenetic relationships to track this ancestry. Consistent with previous surveys using a handful of nuclear genes, an ancestor of taxa in the subtribe Rhytachninae appears to contribute the subgenome related to M1 that retains more genes. Members of this subtribe today live in wet environments in Africa, including taxa growing in river banks such as *Vossia cuspidata* (hippo grass), or seasonally swampy environments like *Oxyrachis gracillima*, *Phacelurus gabonensis*, and *Rhytachne rottboellioides*. The other subgenome donor origin is more obscure, with potential contributions from extant subtribe Ratzeburgiinae and genus *Elionurus*, each of which has a few species present today in the Americas. Where and how this allopolyploidy event occurred is still under investigation. We show that approximately 90% of regions assigned to M1 or M2 based on fractionation are consistent with their phylogenetic origin. Additional analyses are underway to understand how gene conversion events between homeologs may explain the ~10% of regions that disagree. We anticipate this phylogenetic investigation of subgenome ancestry will help maize geneticists interpret the effects of duplicated and fractionated genes within the maize genome. Understanding which subgenome donor is most related to a given maize gene could be of particular utility when considering traits differentiated between these progenitors, such as drought or flooding tolerance and related physiological pathways of cold tolerance.

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P308

Scaling analyses of cis-regulatory evolution across hundreds of wild grass species

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With the rapidly increasing availability of non-model genomes, methods to scale comparative genomic analyses across hundreds or thousands of species are needed. Along with coding sequence changes, non-coding variation underlies much of phenotypic evolution. We investigated cis-regulatory evolution in an extensive panel of Andropogoneae grass species which encompasses diverse environmental adaptations and numerous tropical-temperate transitions. Species were collected globally and used to generate dozens of long-read assemblies and hundreds of additional short read assemblies. We characterized cis-regulatory evolution across Andropogoneae by comparing occurrences of transcription factor (TF) binding motifs across orthologous promoters, testing the hypothesis that TF networks were similarly re-wired during repeated environmental adaptations. We associated presence-absence variation of promoter motifs with species' environmental traits, identifying several TF families for which motif occurrence and/or co-occurrence was strongly associated with environmental variables. The most significant TF families show repeated expansions and contractions of their putative regulons as the Andropogoneae diversified and adapted to new environments. Our approach provides a scalable method to identify TF families that may have been central to adaptation over macroevolutionary time scales, nominating candidates for functional validation.

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P309

Shoot-level phenotyping reveals adaptive variation in the grass genus Hordeum

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After its emergence in the Middle East around 9.5 million years ago, the Hordeum genus, which includes domesticated barley, spread across the Americas and Eurasia, adapting to diverse climatic conditions. The spread of *Hordeum* into new environments was accompanied by rapid speciation and ecological diversification. The Hordeum species are thus an interesting clade to study traits and trait complexes underlying ecological diversification, including life-history variation and local adaptation. In this study, we investigated the phenotypic and genetic variation within and between annual and perennial species of the grass genus *Hordeum*, including the economically important crop barley (*H. vulgare L*). We trialled 56 annual and perennial *Hordeum* accessions, comprising 18 species of the *Hordeum* clade, in a common garden experiment at the University of Düsseldorf, Germany, between the 2021 and 2023 growing seasons. We scored 22 shoot traits at the vegetative and reproductive stages and identified genome-wide genetic variation between and within the species. We tested whether specific climatic variables contributed to phenotypic variation when accounting for geographic distances and phylogenetic structure. Genotype-environment association analyses using redundancy analysis support our hypothesis that local climatic differences contributed to patterns of adaptive divergence among the species across America and Eurasia. Seasonal variation in temperature and precipitation were implicated as primary climatic variables linked to phenotypic variation. We found that variations in plant height, days to flowering, and shoot biomass at harvest are associated with temperature and rainfall seasonality. This study provides insight into genotype-environment interaction and potentially adaptive traits in the Hordeum clade. This work represents a promising starting point for dissecting genetic variation underlying adaptive variation in important life-history traits.

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P310

Tandem repeat structure and conservation in maize

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Centromeres are a critical element of eukaryotic genome architecture,serving as the site of kinetochore assembly and responsible for faithful chromosome segregation during cell division. Specialized histones define functional centromeres epigenetically, but tandemly-repeating DNA is associated on the sequence level. In recent studies of T2T assemblies, a close relationship between tandem repeat structure and function was observed in both human and Arabidopsis genomes. Underlying the functional centromere core exists highly-homogenized repeat patterns. Variation in repeat sequence and copy number among and within genetic lines indicate rapid and recurrent evolution on repeats. Here, we utilize 9 HiFi assemblies to investigate both centromeric and neocentromeric repeat structure. Uniquely, maize has four distinct tandem repeats that relate to centromere (CentC, Cent4) and neocentromere (TR1, knob180) activity. Through comparisons among and within genomes, we evaluate how repeat structure relates to function and how individual arrays in conserved positions vary.

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P311 @Jonatha55038148

The influence of heavy metal response in the origin and domestication of maize

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Domestication is the process of selecting desirable traits over generations, which are identified by the presence of the domestication syndrome. Traits related to development and reproduction are associated with obvious phenotypes. However, adaptive traits were also selected during domestication, albeit not manifested clearly. Therefore, to trace these hidden traits, genetic diversity was compared between maize genotypes and its wild relative, teosinte. Genetic regions mapping near a reported domestication quantitative trait locus (QTL) were found, encoding genes responsive to heavy metals, such as *ZmHMA1* (*Zea mays* heavy metal ATPase1). This gene exhibited selection signatures in upstream and downstream regions, indicating its involvement in domestication. Promoter analysis further revealed that its promoter region had three protein-binding sites for the transcription factor *Tb1*, involved in lateral branching formation, a distinctive trait between maize and teosinte. *ZmHMA1* belongs to a gene family that employs ATP hydrolysis to actively transport substances inside and outside cells. To elucidate the function of *ZmHMA1*, wild maize *W22* plants, *zmhma1* mutant plants, and wild teosinte *parviglumis* plants were grown in soils with sublethal doses of heavy metals, and phenotypic comparisons were made at all stages of development. Surprisingly, the *zmhma1* mutant exhibited vigorous growth and an increased number of seminal roots compared to wild maize, both in the presence and absence of heavy metals. Furthermore, the plants showed constitutive expression of *ZmHMA1* throughout development, regardless of the presence of heavy metals. On the other hand, teosinte *parviglumis* displayed a drastic reduction in lateral branching when grown under high concentrations of heavy metals, resembling a maize phenotype. These results suggest that this gene was affected by domestication, with human selection acting through the regulation pathway of *Tb1*. Allelic variants in maize reduce the activity of *ZmHMA1* compared to teosinte, and exposure to heavy metals could have been a contributing factor to maize domestication

Gene / Gene Models described: *ZmHMA1*; GRMZM2G067853

P312

Uncovering the genetic basis of perenniality in the Andropogoneae

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The Andropogoneae tribe contains some of the most productive C4 grasses relevant to agriculture, including maize, Miscanthus, sorghum, and sugar cane and has adapted to a wide host of environments. Notably, while most of these species 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, 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. We have previously shown that within orthogroups, annual genes are enriched for reduced sequence conservation and premature stop codons, adding support for the loss-of-function hypothesis during perennial-annual transitions. In this study, we further test this hypothesis by leveraging an expanded set of 533 short read and 38 long read Andropogoneae genomes to investigate the genetic basis of perennial-to-annual transitions across the tribe. This set of genomes allows us to test a greater number of transitions and identify instances of repeated loss of function. Using phylogenetic mixed models, we identified genes associated with perenniality across species. Additionally, we identified orthogroups with differential selection constraint in annuals and perennials. Since it is hypothesized that changes in the environment lead to changes in life history, our models were further expanded to include environmental data to determine environmental associations with the perennial growth habit. These models will provide insight into the ecological niches of perennials and annuals. The results from this research will provide a launching point for future work to understand the adaptive potential of perennials and develop maize varieties that are more perennial-like and better adapted for climate change.

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P313

Unraveling the genetic basis of kernel protein concentration in the Illinois long term selection experiment through near-isogenic lines

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The Illinois Long Term Selection Experiment is the longest-running continuous genetics experiment in higher plants. Over 120 years of artificial selection have generated distinct populations representing the phenotypic extremes of kernel protein concentration in maize. To dissect the regions of the maize genome that have led to the rapid and dramatic responses to phenotypic selection, we have derived four populations of near-isogenic lines from combinations of the Illinois Protein Strains. To aid with the phenotypic evaluation of grain protein concentration, these populations also carried the FLOURY2-RFP reporter gene, a visual marker for alpha-zein accumulation that varies dramatically among the Illinois Protein Strains. Kernel protein concentration was measured using both near-infrared reflectance and pink color intensity of the FLOURY2-RFP reporter transgene. Selection for kernel protein concentration was applied throughout the development of these populations to favor genomic regions with functional effect, but not all introgressions displayed phenotypic deviations from the recurrent parent. This observation does not support an "omnigenetic" architecture for selection response, which posits any genomic region harbors loci influencing a complex quantitative phenotype. Leveraging prior work that identified SNPs with evolutionary patterns strongly correlated with changes in kernel protein concentration, our updated investigations of near-isogenic lines help refine and validate candidate genes contributing to the dynamic evolutionary changes in kernel composition.

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P314

Phenotypic selection in SynAdapt, a genetically diverse maize-teosinte hybrid population

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Effective crop improvement methods should be developed with knowledge of the genetic basis of adaptive evolution and an understanding of factors that can limit responses to selection. In particular, well-characterized wild relative and crop systems are ideal for exploring the role of introgression on adaptive evolution because alleles associated with phenotypes under selection can be easily attributed to wild vs. cultivated origin. We are characterizing local phenotypic selection pressures on a maize-teosinte hybrid population (SynAdapt) using a multi-generation local adaptation field experiment at Davidson College. For each of five generations, we will measure the growth, survival, pest-damage, and reproductive success of individual plants. We consider selection on focal traits acting through both female (seed number) and male (total tassel length) components of fitness. Lastly, we will conduct a resurrection experiment using seeds from the first and fifth generations to quantify the extent of adaptive evolution that has occurred. This project will yield valuable data that can be used to teach students about the evolution of complex traits and provides opportunities for students to identify the origin of genetic variants (maize vs. teosinte) associated with fitness components and traits under selection.

P315

Homology of the grass cotyledon: A multiplexed transcriptomic analysis

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A multi-dimensional transcriptomic approach is used to evaluate the homology of grass embryonic organs. Single-cell RNAseq was performed on Stage1 maize embryos, the earliest timepoint when each lateral organ-type of the grass embryo has been initiated. Identification of cell clusters was informed by spatial-RNAseq and laser-microdissection RNAseq of discrete embryonic tissues; correlative spatio-temporal gene-expression models are supported by multiplexed in situ localizations (i.e. RNA-targeting) of 100 embryonic transcripts during key morphogenetic stages in maize embryo ontogeny. The data support a model wherein the single cotyledon of monocotyledonous grasses comprises both the scutellum and the coleoptile. We describe an embryonic-organ-initiation genetic-network that is conserved across taxa and whose expression comprises the phylotypic period of "mid-embryogenesis". We propose an ontogenetic definition of the phylotypic period in place of the chronological description.

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P316

Investigation of Zea mays Ab10 Kindr/Knob180 meiotic drive system in budding yeast

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The meiotic drive system in Zea mays involves the preferential segregation of the abnormal chromosome 10 (Ab10) over the normal chromosome 10 (N10), breaking the laws of Mendelian genetics. Ab10 differs from N10 due to the presence of a heterochromatic knobs that are capable of mobilizing into a neocentromere and causing linked alleles to be inherited at rates up to 80%. Kinesin driver (KINDR) is gene on Ab10 that codes for a kinesin that colocalizes with the knobs, specifically with a DNA sequence called knob180. It is hypothesized that Kindr directly interacts with the knob180 sequence, binding to it and facilitating chromosome movement on meiotic spindles. In addition to KINDR, another candidate gene, SMD13, may contribute to the binding interaction with knob180, as deletion of this gene eliminates the meiotic drive phenotype. Our research is investigating the direct interaction of Kindr and SMD13 candidates with the knob180 sequence via yeast one-hybrid assays. To do this, we are creating transgenic strains containing knob180 DNA repeats, and either Kindr or the SMD13 gene candidates, then performing growth assay on selective media plates. If Kindr directly interacts with knob180 without the assistance from SMD13 or another protein, it will activate the reporter gene and grow on media.

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P317

Characterizing the kinetochore-microtubule binding interface and meiotic spindle structure of Zea mays

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Cell division is contingent on the attachment of microtubules to chromosomes via kinetochores. Zea mays (maize) is an excellent model organism for studying meiosis due to their large chromosomes, ease of gamete dissection, and well-developed cytogenetic methods. Despite this, the structure of the maize meiotic spindle apparatus and kinetochore-microtubule binding interface less characterized than other model systems. Here, we used electron tomography (ET) and the IMOD software to construct 3D models of maize meiotic spindles and their attachment to chromosomes. Imaging revealed an average of 10.8 ± 3.8 kMTS per kinetochore. The size of the maize kinetochore structure was also defined by quantifying the dimensions of the chromatin "cups," the average length and depth being 678 ± 415 nm and 356 ± 235 nm, respectively. Analysis of the meiotic spindle also revealed the presence of small vesicles and large membrane channels. Using immunostaining with polyclonal antibodies to protein disulfide isomerase (PDI), a marker of ER membranes, we found that the channels are positive for PDI indicating an ER origin for these membranes. For future directions, we are continuing to identify novel kinetochore proteins using structural homology with other species and confirmation via immunohistochemistry.

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P318

Regularized AMMI model for multi-environment agricultural trials

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In multi-environment field trials, the additive main effects and multiplicative interaction (AMMI) model is popularly used to analyze yield stability. However, the ordinary AMMI model cannot be used to get predictions for untested genotypes. A novel hierarchical AMMI mixed-effects model for likelihood-based inference is developed that regularizes the genomic main effects and factor scores using kinship information among the genotypes and accommodates the missing data. A scalable stochastic expectation-maximization algorithm is developed for large multi-environment trial datasets, which takes into account possible missing data and is further accelerated by the squared extrapolation method. Simulation studies and maize data from the Genomes to Fields Initiative are used to demonstrate the improvement in the prediction and detection of non-linear genotype-by-environment interactions by the proposed model over the methods available in the literature.

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History of the Maize Genetics Conference

Year	Annual	Location	Dates	Chair
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
1981	23	Allerton Park, IL	March 14-15	Earl Patterson

Year	Annual	Location	Dates	Chair
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

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Notes

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