65th Annual Maize Genetics Meeting

Program and Abstracts

March 16 – 19, 2023

MGC

Maize Genetics Cooperation, Inc.

Facilitated in partnership with

ConferenceDirect®

This conference received financial support from:

National Science Foundation
Bayer
Corteva Agriscience
Syngenta
Resolve BioSciences
KWS SAAT AG
NCGA
BASF
Inari
The Plant Cell
Power Pollen
Genetics Society of America

We thank these sponsors for their generosity!

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

USDA ARS Agricultural Research Service

Table of Contents

Cover image description

Close-up of maize brace roots dripping mucilage after a morning rain. Aside from the roots' function in stabilizing the plant, mucilage can host nitrogen-fixing microbes to benefit the plant.

Cover art by

Jason Wallace
University of Georgia
USA

General Information

Meeting Registration

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

Meals

All meals will be served buffet style in the Midway; serving hours as listed in the Program. Coffee, tea, and soft drinks are available at no charge during the beverage breaks.

Talks and Posters

All Talks will be presented in the Grand Ballroom.

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

The maize meeting is a forum for presentation and discussion of unpublished material. **Photographing or recording of talks and posters is <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 2023 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, follow CDC guidance and if you need assistance, please contact the hotel front desk or contact 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 any posted instructions while 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 out of 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 the Midway, with refreshments and games provided from 9 PM - 11 PM and a cash bar until 1 AM. On Saturday evening there will be informal socializing in the Midway, with refreshments from 9 PM - Midnight, trivia from 10 PM - 11 PM, and a cash bar from midnight - 2 AM.

Access to recorded sessions

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

Steering Committee

Please share your suggestions and comments about the meeting with the 2023 Steering Committee

Matthew Hufford, Chair	(mhufford@iastate.edu)	Ex officio:
Ruben Rellan-Alvarez, co-Chair	(rrellan@ncsu.edu)	Carson Andorf - MaizeGDB
Erin Sparks, Previous Chair	(esparks@udel.edu)	David Braun - Treasurer
Oyenike Adeyemo	(aoadeyemo@unilag.edu.ng)	Darwin Campbell – Planning / Audio Visual
Madelaine Bartlett	(mbartlett@umass.edu)	Marty Sachs - Local Host
Mei Guo	(guomei@kenfeng.com)	John Portwood - Logistics Coordinator
Frank Hochholdinger	(hochhold@uni-bonn.de)	
Sarah Jensen	(sarah.jensen@syngenta.com)	Meeting planning:
Maria Angelica Sanclemente	(sanangelma@gmail.com)	Tricia Simmons – Conference Direct
Aimee Schulz	(ajs692@cornell.edu)	Garrett Simmons – Conference Direct
Petra Wolters	(petra.wolters@corteva.com)	
Marna Yandeau-Nelson	(myn@iastate.edu)	

Acknowledgements

Many thanks go to Carson Andorf, John Portwood, and the MaizeGDB staff from the USDA-ARS, as well as 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 a multitude of other issues. Special thanks are also extended to the Union Station staff for their help in organizing this conference. Thanks go to Mei Guo, Sarah Jensen, 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 65th Maize Genetics Meeting.

From the Maize Genetics Cooperation Board of Directors

Maize Genetics Awards:


The 2023 MGC Cooperator Awardees

Carolyn Lawrence-Dill, Iowa State University
Bob Meeley, DuPont-Pioneer (retired)

The 2023 MGC Leadership Awardee

John Fowler, Oregon State University

The 2023 M. Rhoades Early Career Awardee

Marna Yandeau-Nelson, Iowa State University

The 2023 L. Stadler Mid-Career Awardee

David Braun, University of Missouri


The 2023 R. Emerson Lifetime Awardees

Hugo Dooner, Rutgers University
Kathy Newton, University of Missouri

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 **2023** Barbara McClintock Prize for Plant Genetics and Genome Studies has been awarded to Dr. Virginia Walbot who will present a McClintock Prize Address on Friday, March 17, 8:10pm CDT (See Page 26).

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

NSF-funded Research Coordination Network for maize genetics:

The National Science Foundation is supporting a 5-year Research Coordination Network project titled "Broadening and Energizing the Maize Research Community". The project began in January, 2018, and is coordinated by the Maize Genetics Advocacy Committee. The grant funds activities at the Maize Genetics Meeting including the MaGNET program and travel awards to increase disciplinary breadth and underrepresented participation. In addition, the funding allows the Maize Genetics Meeting to systematically enrich the program during the term of the grant. Mid-year conferences are planned yearly to focus on specific topics that are important to the community. The first mid-year conference was held in Madison, WI in September 2018 and included an overall visioning session as well as focus on Functional Genomics Tools and Resources. The second mid-year conference was held in Madison, WI in September 2019 and focused on Data Collection and Curation, Databases, and Genome Annotation. The third mid-year virtual conference was held in October 2020 and focused on public-private partnerships and career development. The fourth mid-year virtual conference was held in January 2022 and focused on strategies for a healthy and inclusive research community. White papers summarizing conclusions of the mid-year conferences are available on MaizeGDB (https://www.maizegdb.org/mgc/advocacy/docs.php). Teams have been assembled within the RCN to focus on: Functional Genomics Tools and Resources; Informatics Tools, Resources, and Services; Training and Student Recruitment; Developing Country Interface and Community Breadth; Industry Interface; and diversity, equity, and inclusion within the maize community. 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 2023 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.

2023 MaGNET Awardees

Undergraduate

Katherine Gray, University of Florida
Simone Murguia, University of Hawaii
Blessing Ngara, Iowa State University ..... Poster #84

Graduate Student

Boris M. E. Alladassi, Iowa State University ..... Poster #157
Comfort Bonney Arku, University of Massachusetts ..... Poster #27
Andrew Egesa, University of Florida ..... Poster #19
Mercy Fakude, Iowa State University ..... Poster #171
Lina Gomez-Cano, Michigan State University ..... Poster #268
Irene Ikiriko, University of Delaware ..... Poster #18
Kimberly Rispress, East Carolina University ..... Poster #10
Ruthie Stokes, North Carolina State University ..... Poster #265
Nirwan Tandukar, North Carolina State University ..... Poster #236
Destiny Tyson, North Carolina State University ..... Poster #155

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

Primarily Undergraduate Institutions and Disciplinary Breadth Awards

Primarily Undergraduate Institutions (PUI) and Disciplinary Breadth (DB) are two financial aid programs that seek to recruit and retain scientists from PUIs and plant-related disciplines into the maize research community by encouraging their attendance at the Annual Maize Genetics Meeting (MGM). The PUI program seeks to welcome students and faculty from Primarily Undergraduate 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 PUI or DB award.

2023 PUI Awardees

Student

Terice Kelly, Mount Holyoke College ..... Poster #32

Faculty

Irina Makarevitch, Hamline University ..... Poster #121

2023 DB Awardees

Student

Jyothi Prasanth, Iowa State University ..... Poster #104
McKena Wilson, Michigan State University ..... Talk #9
Colleen Yanarella, Iowa State University ..... Poster #99

Postdoc

Sanbon Gosa, University of Illinois
Ha Duong, University of Missouri ..... Poster #293

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

Broadening International Participation Awards

The 2023 Broadening International Participation Award program seeks to promote international attendance for researchers from countries that are historically under-represented at the Maize Meeting. This 2023 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.

Faculty
Soon-Kwon Kim
Mohammad Ismail

Research Scientists
Muhammad Zafar Iqbal
Usman Aslam
Isabela Figueiredo de Oliveira

Graduate Students
Olayinka Murtala Ashiru
Varalakshmi S
Georgina Lala Ehemba

FAIR Data Management

...A Reminder from the MaizeGDB team

MaizeGDB integrates large amounts of published data so our community can find and use it easily. Your efforts to make your data Findable, Accessible, Interoperable and Reusable (FAIR; go-fair.org) allow MaizeGDB and others to integrate even more data. Here are basic guidelines for FAIR DATA management for you to apply to data you have generated, and to data in papers and grants that you review.

An example of an outstanding FAIR data paper is:
Savadel SD, Hartwig T, Turpin ZM, Vera DL, Lung PY, Sui X, Blank M, Frommer WB, Dennis JH, Zhang J, Bass HW. **The native cistrome and sequence motif families of the maize ear.** PLoS Genet. 2021 Aug 12;17(8):e1009689. doi: 10.1371/journal.pgen.1009689. PMID: 34383745; PMCID: PMC8360572.

Note the Supporting Information has even BED and bigwig files! These were easy to directly incorporate into the MaizeGDB Genome Browser. We encourage authors to take this much care with their data- to help consolidate information we have on each gene, each protein, each genome, etc.!

- **Put your Data in the right Database.** For example, DNA/RNA/Protein Sequences, genome assemblies and annotations should go to the long term repositories like NCBI. Report the accession numbers in your paper. All maize SNPs should be submitted to EVA at EBI. (https://www.ebi.ac.uk/eva/?Submit-Data). See more repositories at maizegdb.org/FAIRpractices and journal websites
- **Publish the data with the paper.** If your journal article refers to big data NOT published with your article, please make sure to obtain, from the data repository where you put your data, a persistent identifier like a DOI number, and add that to your article. You can publish datasets alone in journals like https://www.micropublication.org, just make sure to link data with the paper describing it. When reviewing papers, please ensure reported data is actually present and FAIR.
- **Use established unique identifiers for genes, gene models, genomes, etc.** Don't rename genes that already have names. For gene names, please look up your gene name symbol at MaizeGDB. If reporting on a gene sequence, please use the **exact gene model ID** (which will also ID the genome from which it came). If there is no gene model for your gene, please deposit your sequence at NCBI, and report the NCBI identifier in your paper. If reporting on a protein, please use the correct ID from NCBI or UniProt. If it's not there, please submit your protein sequence to NCBI or Uniprot.
- **Attach complete and detailed metadata to your data sets, and use accepted file formats.** When you deposit data, you are asked for information about your data (metadata). Please give this the same careful attention you give to your bench work and analysis. Datasets that are not adequately described are not reusable or reproducible, and raise questions about the quality of the research.
- **Ensure data sets are "machine readable".** When describing data, use permanent identifiers wherever possible, use the proper case (LG1 is not the same as lg1), and include GO, PO, PATO terms when possible. Please check and validate that your data is in common, well-used machine readable formats.
- **Budget time for Data Management.** Please budget time to do a good job of managing your data as you are with the other aspects of your research.
- **Familiarize yourself with the FAIR data sharing 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
For more information on FAIR principles visit: https://www.maizegdb.org/FAIRpractices

What's NEW at MaizeGDB!

In 2022, 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:

- 99 genomes
- Over 2 million gene model 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
- 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
- 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 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 2022 MaizeGDB Editorial Board Members!
Mohammad Arif Ashraf (Year 3!!), PostDoc, UMass Amherst, MA
Kaitlin Higgins, Graduate Student, Iowa State University, Ames, IA
Beibei Liu, Graduate Student, Miami University, Oxford, OH
Hai Wang, Faculty, China Agricultural University, China
Lei Liu, Faculty, Huazhong Agricultural University, China
Lander Geadelmann, Graduate Student, Iowa State University, Ames, IA
Anuradha Singh, Postdoc, Michigan State University

Welcome 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

Continuing on in 2023 for the 2nd year – Editorial Board DEI Papers recommended by CODIE Editors!
Andrew Egesa, Graduate Student, University of Florida, Gainesville, FL
Tessa Durham Brooks, Faculty, Doane University, Crete, NE

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 TAIR, FlyBase, WormBase, PomBase, and now MaizeGDB**! Thus, *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 this 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 (see https://www.maizegdb.org/FAIRpractices). Upon acceptance, your article is curated into MaizeGDB: coupling publication with curation and discoverability in MaizeGDB. The cost to publish is only $250.

Here are some example publications:

New Finding: Oh S, Kong Q, Montgomery BL. Guard-cell phytochromes impact seedling photomorphogenesis and rosette leaf morphology. MicroPubl Biol. 2022 Jan 31;2022. doi: 10.17912/micropub.biology.000521. PMID: 35128344; PMCID: PMC8808294.

Materials and Reagents: Marques J, Matiolli CC, Abreu IA. Visualization of a curated *Oryza sativa* L. CDPKs Protein-Protein Interaction Network *(*CDPK-OsPPIN*)*. MicroPubl Biol. 2022 Jan 26;2022. doi: 10.17912/micropub.biology.000513. PMID: 35098050; PMCID: PMC8792674.

Negative Results: Martineau CN, Maynard CA, Pujol N. ATFS-1 plays no repressive role in the regulation of epidermal immune response. MicroPubl Biol. 2022 Feb 22;2022. doi:10.17912/micropub.biology.000525. PMID: 35224461; PMCID: PMC8864481.

For more information:

Visit the journal: https://www.micropublication.org

Read this article: Raciti D, Yook K, Harris TW, Schedl T, Sternberg PW. Micropublication: incentivizing community curation and placing unpublished data into the public domain. *Database (Oxford)*. 2018;2018:bay013. doi:10.1093/database/bay013

Or talk to Carson Andorf at MaizeGDB, https://www.maizegdb.org/contact

SCHEDULE OF EVENTS

Talks will be held in the Grand Ballroom

Posters will be displayed in the Midway

Thursday, March 16, 2023

9:00 AM – 6:00 PM	**OPTIONAL PRE-CONFERENCE WORKSHOPS**	
9:00 AM – 4:00 PM	**Maize Development and Cell Biology Workshop** Space is limited, registration is required	Midway Suites 1-4
1:00 PM - 2:00 PM	**MaizeMine Data Warehouse Tutorial**	Jeffersonian/ Knickerbocker
2:00 PM - 3:00 PM	**Gramene: Maize Pan-genome Resources**	Jeffersonian/ Knickerbocker
2:00 PM - 4:30 PM	**Tour of Donald Danforth Plant Science Center** Space is limited, registration is required Transportation provided, meet at Union Station front desk	
3:15 PM - 3:45 PM	**MaizeGDB: Protein Structure Resources**	Jeffersonian/ Knickerbocker
3:45 PM - 4:45 PM	**MaizeGDB: Pan-genome Resources and Visualization**	Jeffersonian/ Knickerbocker
3:00 PM – 9:30 PM	**REGISTRATION** (Depot Registration Office)	
3:00 PM – 6:00 PM	**POSTER HANGING** (Midway)	
5:00 PM – 5:45 PM	**MaGNET Awardees and Mentors Introductions**	Midway Suites 1-4
6:00 PM – 7:00 PM	**DINNER** (Midway)	

Thursday, March 16, 2023 (continued)

7:00 PM – 9:00 PM	**SESSION 1 – WELCOME / THE GENES THAT MAKE MAIZE** Chair: Matthew Hufford	Talks 1-4.
7:00 PM	**WELCOME AND ANNOUNCEMENTS**	(Grand Ballroom)
7:15 PM	**Hua Yang, University of Missouri** *Identification of a trans-acting factor required for B chromosome nondisjunction-a component of its drive mechanism*	[T1]
7:35 PM	**Hui Liu, University of Florida** *Distinct roles of plastidial and cytosolic arogenate pathways for phenylalanine and tyrosine biosynthesis in kernel and plant development*	[T2]
7:55 PM	**Michaela Matthes, University of Bonn** *Genome-wide association study identifies a link between boron homeostasis and benzoxazinless3 in maize*	[T3]
8:15 PM	**Siddique Aboobucker, Iowa State University** *Parallel spindle genes restore haploid male fertility – removing a bottleneck in doubled haploid technology*	[T4]
8:35 PM	**Poster Lightning Talks**	
9:00 PM – 10:00 PM	**UNDERGRADUATE AND GRADUATE STUDENT MIXER**	(Pegram)
9:00 PM – 1:00 AM	**INFORMAL POSTER VIEWING & HOSPITALITY**	(Midway)

Friday, March 17, 2023

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

7:30 AM – 12:30 PM **REGISTRATION** (Depot Registration Office)

8:00 AM – 10:10 AM **SESSION 2 – CEREALS AND THE ENVIRONMENT**
Chair: Marna Yandeau-Nelson Talks 5-9.

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

8:15 AM **Aaron Kusmec, Iowa State University** [T5]
A genetic tradeoff for tolerance to moderate and severe heat stress in US hybrid maize

8:35 AM **August Thies, Donald Danforth Plant Science Center** [T6]
Analyzing the impact of breeding, soil variability and management practices on maize root system architecture using X-ray imaging

8:55 AM **Vladimir Torres, University of Nebraska-Lincoln** [T7]
Measurement of expression from a limited number of genes is sufficient to predict flowering time in maize

9:15 AM **Anuradha Singh, Michigan State University** [T8]
Genetic analysis of leaf functional and eco-physiological traits for optimized photosynthesis in sorghum

9:35 AM **McKena Wilson, Michigan State University** [T9]
Quantitative genetics of stress tolerance and agronomic traits in the climate resilient cereal teff

9:55 AM **Poster Lightning Talks**

10:10 AM – 10:40 AM **BREAK** **Foyer A**

10:40 AM – 12:30 PM **SESSION 3 – INVITED SPEAKERS**
Chair: Petra Wolters

10:40 AM Introduction

10:50 AM **Ralph Bock, Max Planck Gesellschaft** [IS1]
Genetic engineering of chloroplast and mitochondrial genomes

11:40 AM **Candice Hirsch, University of Minnesota** [IS2]
From genome to phenome: Understanding the diversity of maize

Friday, March 17, 2023 (continued)

12:30 PM – 1:30 PM **LUNCH** (Midway)
MaGNET/PUI Networking Lunch (Midway Suites 1&2)
MGC BoD and MGAC Lunch (Midway Suites 3&4)

1:30 PM – 4:30 PM **POSTER SESSION 1** (Midway)

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

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

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

4:40 PM – 6:00 PM **SESSION 4 – PREDICTING MAIZE PERFORMANCE**
Chair: Aimee Schulz Talks 10-12.

4:40 PM **Zhikai Yang, University of Nebraska-Lincoln** [T10]
Microbiome-enabled genomic selection improves prediction accuracy for nitrogen-related traits in maize

5:00 PM **Daniel Kick, USDA-ARS, University of Missouri** [T11]
Maize yield prediction accuracy increased by inclusion of genetics, environment, and management interactions with deep learning

5:20 PM **Alencar Xavier, Corteva, Purdue University** [T12]
Maize yield prediction: Results from the 2022 G2F prediction competition

5:40 PM **Poster Lightning Talks**

6:00 PM – 7:00 PM **DINNER** (Midway)
Bayer Student/Postdoc Dinner (Midway 1&2)

7:00 PM –9:00 PM **SESSION 5 – AWARDS & McCLINTOCK PRIZE PRESENTATION**

7:00 PM **Paula McSteen, University of Missouri and Hank Bass, Florida State University**
Presenting: Cooperator and Leadership Awards

7:20 PM **Andrea Eveland, Donald Danforth Plant Science Center**
Presenting: M. Rhoades Early-Career, L. Stadler Mid-Career

7:40 PM **Jay Hollick, The Ohio State University**
Presenting: R. Emerson Lifetime Awards

8:00 PM **Mark Lubkowitz, Saint Michael's College**
McClintock Prize Presentation Introduction

8:10 PM **McClintock Prize Presentation: Virginia Walbot**
Development of maize anthers: it's a long road from lobe inception to functional pollen

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

Saturday, March 18, 2023

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

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

8:00 AM – 10:00 AM **SESSION 6 – CELLULAR PROCESSES**
Chair: Madelaine Bartlett Talks 13-18.

8:00 AM **Hao Wu, Cornell University** [T13]
Spatial transcriptomic analysis of the maize embryo

8:20 AM **Hardy Rolletschek, Leibniz Institute, IPK** [T14]
The peripheral (maternal) void network inside the maize kernel supports grain fill

8:40 AM **Jazmin Abraham-Juarez, Langebio Cinvestav** [T15]
Liguleless narrow and narrow odd dwarf regulate maize development and stress response in overlapping pathways

9:00 AM **Ruthie Angelovici, University of Missouri** [T16]
Uncovering the genetic and metabolic bases of seed amino acid composition using a multi-omics integration approach

9:20 AM **Mateusz Zelkowski, Cornell University** [T17]
The meiotic crossover landscape in maize

9:40 AM **Jeff Chen, The University of Texas at Austin** [T18]
Circadian Regulation of Metabolomes and Proteomes in Maize Heterosis

10:00 AM – 10:40 AM **BREAK, POSTDOC MIXER** **Foyer A**

10:40 AM – 12:30 PM **SESSION 7 – INVITED SPEAKERS**
Chair: Matthew Hufford

10:40 AM Introduction

10:50 AM **Seung Yon (Sue) Rhee, Carnegie Institution for Science** [IS3]
Understanding mechanisms of thermoadaptation of desert extremophiles

11:40 AM **Damon Lisch, Purdue University** [IS4]
Local and global perspectives on the causes and consequences of epigenetic silencing of transposable elements

12:30 PM – 1:30 PM **LUNCH** (Midway)
MaGNET Lunch with Invited Speakers (Midway Suites 1&2)
MGMSC Meeting (Midway Suites 3&4)
Maize Genetics Mentoring Program (Midway 11)
Networking Lunch

Saturday, March 18, 2023 (continued)

1:30 PM – 4:30 PM **POSTER SESSION 2** (Midway)

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

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

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

4:30 PM – 6:00 PM **COMMUNITY SESSION - Maize Genetics Cooperative**
Jay Hollick, MGC BoD Chair (Grand Ballroom)

6:00 PM – 7:00 PM **DINNER** (Midway)
Corteva Student/Postdoc Dinner (Midway 1&2)
Syngenta Student/Postdoc Dinner (Midway 3&4)

7:00 PM – 8:20 PM **SESSION 8 – EVOLUTION OF THE MAIZE GENOME**
Chair: Rubén Rellán Álvarez Talks 19-22.

7:00 PM **Jeffrey Ross-Ibarra, University of California, Davis** [T19]
Two teosintes made modern maize

7:20 PM **Manisha Munasinghe, University of Minnesota** [T20]
Combined analysis of transposable elements and structural variation in maize genomes reveals genome contraction outpaces expansion

7:40 PM **John Pablo Mendieta, University of Georgia** [T21]
Deciphering the evolutionary history of cell-type-specific accessible chromatin regions by comparative genomic approaches

8:00 PM **Michelle Stitzer, Cornell University** [T22]
Elevated transposable element content is subtly associated with reduced fitness in maize

8:20 PM – 8:40 PM **BREAK** **Foyer A**

8:40 PM – 9:30 PM **SESSION 9 – FOSTERING DIVERSITY IN THE MAIZE COMMUNITY**
Chair: Maria Angelica Sanclemente

8:40 PM **MariaElena Zavala, California State University, Northridge** [IS5]
Sowing the seeds of equity to reap a harvest of diverse scientists

9:30 PM – 2:00 AM **INFORMAL POSTER VIEWING, & HOSPITALITY** (Midway)

10:00 PM – 11:00 PM **TRIVIA!!** (Pegram)

Sunday, March 19, 2023

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

Posters should be taken down by 9 AM!

8:20 AM – 10:00 AM **SESSION 10 – EMERGING TOOLS AND APPLIED RESEARCH**
Chair: Sarah Jensen Talks 23-27.

8:20 AM **Aimee Schulz, Cornell University** [T23]
reelGene: Fishing for good gene models with evolution and machine learning

8:40 AM **Marco Peixoto, University of Florida** [T24]
An R-package for cross prediction and optimization using genomic selection

9:00 AM **Samik Bhattacharya, Resolve Biosciences** [T25]
Single-cell transcript mapping in crop species using Molecular Cartography™

9:20 AM **Xianran Li, USDA-ARS, Washington State University** [T26]
Streamline unsupervised machine learning to survey and graph indel-based haplotypes from pan-genomes

9:40 AM **Preston Hurst, University of Nebraska-Lincoln** [T27]
Editing the 19kDa alpha-zein gene family generates non-opaque2 based quality protein maize

10:00 AM – 10:30 AM **BREAK** **Foyer A**

10:30 AM – 11:40 AM **SESSION 11 – REGULATING GENES AND GENOMES**
Chair: Erin Sparks Talks 28-30.

10:30 AM **Emily Wear, North Carolina State University** [T28]
Metabolic labeling of nascent RNA: a look into active transcription and stable vs. unstable transcripts in maize root tips

10:50 AM **Julien Rozière, Université Paris-Saclay, CNRS, INRAE** [T29]
Detection of preferentially located DNA motifs reveals distinct cis-regulatory sequences in gene-proximal regions of Arabidopsis thaliana and maize

11:10 AM **Nicholas Gladman, USDA-ARS, Cold Spring Harbor** [T30]
Direct and predicted motif analysis of GRAS family transcription factors in sorghum and other important crop species

11:30 AM **CLOSING REMARKS (Matthew Hufford and Rubén Rellán Álvarez)**

11:40 AM **ADJOURNMENT**

Poster List

Cell and Developmental Biology

P1	**Michael Scanlon** <mjs298@cornell.edu>	*A WOX3-patterning module organizes planar growth in grass leaves and ligules.*
P2	**George Chuck** <georgechuck@berkeley.edu>	*A developmental boundary built by mutual microRNA mediated repression*
P3	**Xiaosa Xu** <xxu@cshl.edu>	*A nuclear moonlighting function of RAMOSA3 in maize inflorescence branching*
P4	**Arif Ashraf** <arif.ashraf.opu@gmail.com>	*A polarized nuclear position is required for correct division plane specification during maize stomatal development*
P5	**Vai Lor** <vslor@wisc.edu>	*Agrobacterium-mediated transformation of sweet corn inbred lines using morphogenic genes*
P6	**Cristina Guerrero Méndez** <cristina.guerrerom@cinvestav.mx>	*Analysis of FUN1 protein for identification of new factors involved in sex determination in maize*
P7	**Pablo Silva-Villatoro** <pablo.silvav@cinvestav.mx>	*Analysis of NARROW ODD DWARF in maize immune response*
P8	**Aimee Uyehara** <auyeh002@ucr.edu>	*Analysis of aberrant, preprophase band-independent TANGLED1 recruitment to the cell cortex in maize*
P9	**Olivia Hazelwood** <oliviaha@udel.edu>	*Characterization of mechanosensitive MSL gene family expression in Zea mays aerial and ground brace roots*
P10	**Kimberly Rispress** <rispressk04@students.ecu.edu>	*Characterization of the floral development mutant Polytypic ear1*
P11	**Samuel Leiboff** <leiboffs@oregonstate.edu>	*Comparing hormone dynamics in cereal crops via transient expression of hormone sensors*
P12	**Penelope Lindsay** <lindsay@cshl.edu>	*Control of maize ear development by a CLAVATA-related receptor complex*
P13	**Edoardo Bertolini** <ebertolini@danforthcenter.org>	*Cross-species analysis of gene regulatory circuitries underlying early inflorescence development in Panicoid grasses*
P14	**Rodrigo Muñoz Javier** <rodrigo.munoz@cinvestav.mx>	*Deciphering the molecular function of Sympathy for the ligule (Sol1) in development and maize immune response*
P15	**Zongliang Chen** <zlchen@waksman.rutgers.edu>	*Dissecting cis-regulatory control of ZmWUS1 expression through the binding of type-B response regulator proteins*
P16	**Clinton Whipple** <whipple@byu.edu>	*Diverse mechanisms of tiller suppression in domesticated setaria and maize*
P17	**Alec Chin-Quee** <chinquee2@ufl.edu>	*Diversifying selection in evolution of the pleiotropic BIGE7/BRIZ E3-ligase*
P18	**Irene Ikiriko** <iikiriko@udel.edu>	*Does stalk flexural stiffness require cellular-scale mechanosensing?*
P19	**Andrew Egesa** <egesaa@ufl.edu>	*Effect of heat stress on reproductive development in Zea mays L*
P20	**Rachel Egger** <rachel.egger@syngenta.com>	*Electric field treatment induces chromosome doubling in maize cell culture*
P21	**Héctor H. Torres-Martínez** <hhtormar@stanford.edu>	*Engineering root system architecture to improve teh water use efficiency of grasses*
P22	**Willian Goudinho Viana** <viana@stanford.edu>	*Exploring a new pathway for crown root development in grasses*

P23	**John Hodge** <jgerardhodge@gmail.com>	*Function-value trait modeling of computer vision-derived epidermal cell patterning data to reveal the genetic basis of stomatagenesis in C4 grasses*
P24	**John Fowler** <fowlerjo@oregonstate.edu>	*Functional interrogation of pollen-expressed genes using the Ds-GFP insertional mutant population*
P25	**Yuguo Xiao** <yxiao@danforthcenter.org>	*Genetic basis of tillering in sorghum*
P26	**Amina Chaudhry** <amina.chaudhry@rutgers.edu>	*Genetic dissection of maize shoot and inflorescence architecture*
P27	**Comfort Bonney Arku** <cbonneyarku@umass.edu>	*Genetic networks underlying unisexual flower development in maize*
P28	**Tej Man Tamang** <tejman@ksu.edu>	*Identification of candidate callus regenerative genes using genetic mapping and transcriptional profiling*
P29	**Jessica Ji** <jjessica@iastate.edu>	*Improved maize transformation service by Crop Bioengineering Laboratory at Iowa State University*
P30	**Minjeong Kang** <mjkang@iastate.edu>	*Improving altruistic transformation system for gene editing of public maize genotypes*
P31	**Josh Strable** <jjstrabl@ncsu.edu>	*Integrating ethylene signaling into maize domestication regulatory pathways*
P32	**Terice Kelly** <tmkelly@umass.edu>	*Investigating the role of RAMOSA3 (RA3) in grass flower development and evolution*
P33	**Stephanie Martinez** <smart046@ucr.edu>	*KATANIN is required for microtubule severing, growth and division positioning in maize*
P34	**Jacob Brunkard** <brunkard@wisc.edu>	*Liguleless narrow and narrow odd dwarf act in overlapping pathways to regulate maize development and metabolism*
P35	**Thanduanlung Kamei** <thanduan@udel.edu>	*Linking brace root development to function*
P36	**David Zimmerman** <djz3@illinois.edu>	*Maize Kernel Grafting: A simplified method*
P37	**Lander Geadelmann** <landerg@iastate.edu>	*Maize developmental transcription factors that regulate leaf initiation, morphology, and phyllotaxy*
P38	**Jada Smith** <jspmf@umsystem.edu>	*Maize Cpd6 encodes a sucrose synthase hypothesized to uniquely function in cell wall development in the phloem*
P39	**Michael Busche** <busche2@wisc.edu>	*Mapping the maize TOR signaling network*
P40	**Jason Gregory** <jason.gregory@rutgers.edu>	*Master Meristem Manipulators: Regulation of meristem size by the REL2 corepressor family*
P41	**Xia Zhang** <xia.zhang@syngenta.com>	*Molecular regulation for improving maize androgenesis*
P42	**Gergely Motolai** <gmotolai@ksu.edu>	*Monothiol glutaredoxin AtGRXS17-expressing maize plants increase yield under combined heat and drought stress*
P43	**Mercy Azanu** <mkazanu@iastate.edu>	*Morphogenic genes assisted Agrobacterium-mediated transformation of tropical maize genotypes*
P44	**Diana Ruggiero** <ruggiedi@oregonstate.edu>	*Quantitative genetics and high-throughput phenotyping of maize leaf vascular traits*
P45	**Thu Tran** <tran@cshl.edu>	*RAMOSA3 interacts with catalytic and non-catalytic TREHALOSE-6-PHOSPHATE SYNTHASES (ZmTPSs) to control embryo and inflorescence development*
P46	**Junpeng Zhan** <jzhan@danforthcenter.org>	*Regulation and evolution of reproductive phasiRNAs in maize and related species*

P47	**Craig Cowling** <ccowling@iastate.edu>	*Roles of auxin transporter PILS6 in maize growth and development*
P48	**Erin Sparks** <esparks@udel.edu>	*SMURF: A new tool to measure root torsional stiffness for understanding root lodging-resistance*
P49	**Taylor Clarke** <tlclarke@stanford.edu>	*Screening maize orthologous genes in Arabidopsis thaliana for defects in moisture-regulated root branching*
P50	**Lukas Evans** <Le95@cornell.edu>	*Single cell RNA-seq and confocal imaging analyses of blade-sheath boundary development in the maize leaf.*
P51	**Xiaosa Xu** <xxu@cshl.edu>	*Single-cell analysis of plant shoot meristems opens a 'goldmine' for functional studies*
P52	**Hao Wu** <hw388@cornell.edu>	*Spatial transcriptomic analysis of the maize embryo*
P53	**Linkan Dash** <linkan@iastate.edu>	*The Auxin Response Factor ARF27 is required for maize root morphogenesis*
P54	**Hailong Yang** <hailongyang@umass.edu>	*The cis-regulatory evolution of GRASSY TILLERS1 (GT1) over deep time*
P55	**Zongliang Chen** <zlchen@waksman.rutgers.edu>	*The combination of morphogenic regulators BABY BOOM and GRF-GIF improves maize transformation efficiency*
P56	**Prameela Awale** <pa96f@mail.missouri.edu>	*The enhancer of spi1 (eos1) gene is involved in auxin regulated maize inflorescence development*
P57	**Blaine Marchant** <dbmarchant@gmail.com>	*The establishment of the anther somatic niche with single cell sequencing*
P58	**Fernanda Ghenov** <fghenov@hawaii.edu>	*The flowering phenotypes of temperate and tropical maize grown in short and long day field environments*
P59	**Sessen Daniel Iohannes** <iohannes@cshl.edu>	*The redundancy paradox: uncovering the mechanisms of active compensation between paralogous genes in the maize meristem*
P60	**Lily O'Connor** <loconnor@danforthcenter.org>	*Trans-activation analysis of maize anther transcription factors using protoplasts*
P61	**Amber de Neve** <adeneve@umass.edu>	*Trehalose-6-phosphate-phosphatases have a conserved role shaping Panicoid inflorescence architecture*
P62	**Alejandro Aragon-Raygoza** <jaaragon@ncsu.edu>	*Untangling the effects of ethylene in maize vegetative shoots through single-cell transcriptomics*
P63	**Brian Zebosi** <bzebosi@iastate.edu>	*bds1 and bds2 redundantly regulate inflorescence and shoot architecture in maize via brassinosteroid biosynthesis*
P64	**Colin Wilburn** <Crw8hn@umsystem.edu>	*lateral rootless2 (lrs2) functions in maize root development*

Computational and Large-Scale Biology

P65	**Carson Andorf** <carson.andorf@usda.gov>	*MaizeGDB: Maize protein structure resources*
P66	**Ethalinda Cannon** <Ethy.Cannon@usda.gov>	*Pan-genome data at MaizeGDB*
P67	**Jack Gardiner** <jack.m.gardiner@gmail.com>	*MaizeMine: New tools for Zea mays pangene data mining*
P68	**Rita Hayford** <ritakusiappiah@gmail.com>	*Stress response functional annotation using RNA expression in maize*
P69	**Shatabdi Sen** <shatabdi@iastate.edu>	*Maize Feature Store (MFS): A centralized resource to manage and analyze curated maize multi-omics features for machine learning applications.*

P70	**Jonathan Turkus** <jturkus2@unl.edu>	*A common resequencing-based genetic marker dataset for global maize diversity*
P71	**Olivier Niyonshuti Mizero** <niyomizer@huskers.unl.edu>	*A deep learning object detection pipeline enables rapid detection of maize kernels*
P72	**Jesse Hickman** <jhickman@iastate.edu>	*A time-series analysis of Azimuthal Canopy Orientation Architecture using UAV images*
P73	**Anna Pardo** <haberan2@msu.edu>	*An improved genome assembly and annotation of the tetraploid resurrection grass Eragrostis nindensis*
P74	**Cinta Romay** <mcr72@cornell.edu>	*An improved maize Practical Haplotype Graph (PHG) to study and store maize diversity*
P75	**Tim Kosfeld** <TKosfeld@danforthcenter.org>	*Building a practical haplotype graph based genetic map of the zea synthetic population*
P76	**Keting Chen** <kchen@iastate.edu>	*Characterization of the gene networks underlying cuticle production in maize silks via systems biology approaches*
P77	**Brandon Monier** <bm646@cornell.edu>	*Cloudifying reproducible breeding pipelines: integrating TASSEL and the PHG with R and JupyterHub*
P78	**Allen Hubbard** <ahubbard@danforthcenter.org>	*Combining GWAS of metabolomic and transcriptomic datasets to study the impact of drought on plant growth and metabolism in Sorghum and Setaria*
P79	**Emily Wheeler** <eamarkha@ncsu.edu>	*Comparative genomic analysis of DNA replication in maize and sorghum*
P80	**Jordan Manchego** <manchego@msu.edu>	*Comparison of methods for detection and quantification of tar spot foliar infection in maize using dynamic colorspace thresholding, object detection, and contour analysis*
P81	**Leigh Mickelson-Young** <lamickel@ncsu.edu>	*DNA replication timing: A comparison of two genomic methods in maize root tips*
P82	**Divya Mishra** <dmishra5@wisc.edu>	*Deciphering the gene regulatory network underlying compensatory mechanisms of autophagy in maize*
P83	**Brandon Webster** <webst250@msu.edu>	*Field and transcriptomic approach to understand hybrid specific response to N supplementation*
P84	**Blessing Ngara** <ngarable@iastate.edu>	*GO-based comparative functional analysis of three maize genomes*
P85	**Leila Fattel** <lfattel@iastate.edu>	*Gene ontology based comparative functional genomics in plants*
P86	**Yan Zhou** <yzhou86@iastate.edu>	*Genetic regulation of self-organizing canopy patterns and their impacts on light interception in maize*
P87	**Zhenyuan Lu** <luj@cshl.edu>	*Gramene PanMaize: One-stop pan-genome browser for exploring the rich genetic diversity in maize*
P88	**Doreen Ware** <doreen.ware@usda.gov>	*Gramene: Comparative plant genome resource*
P89	**Colin Finnegan** <cpf@iastate.edu>	*Growing up: How the UTR environment alters as genes age*
P90	**Musa Ulutas** <ulutas.musa@huskers.unl.edu>	*High throughput phenotyping of field excavated roots for diverse maize inbred and hybrid lines under different N conditions.*
P91	**Cassandra Palmer** <cpalmer9@unl.edu>	*High-throughput phenotypic analysis of plant growth in lunar regolith simulant and the potential applications in space agriculture*
P92	**Zong-Yan Liu** <zl843@cornell.edu>	*Identification of seed storage proteins across andropogoneae genomes with machine learning*

P93 **Travis Wrightsman** <tw493@cornell.edu> — *Improving sensitivity of variant effect estimates on RNA expression through cross-species training*

P94 **Zehta Glover** <zglover@fsu.edu> — *MNase sequence bias fails to predict MOA-seq chromatin profiling peaks*

P95 **Alexandria Tran** <tran30@illinois.edu> — *Maize gene expression responses to late season application of various nitrogen sources*

P96 **Yuxiang Guo** <yxguo@iastate.edu> — *Maize orphan genes and their potential association with cuticle synthesis*

P97 **Andrea Gallavotti** <agallavotti@waksman.rutgers.edu> — *Mapping and functional characterization of cis-regulatory variation in maize*

P98 **Ally Schumacher** <schum193@msu.edu> — *Multi-omic analyses of maize color and leaf traits*

P99 **Colleen Yanarella** <cfy@iastate.edu> — *Proof of concept for spoken natural language descriptions of phenotypes for association genetics applications*

P100 **Janeen Braynen** <braynen@cshl.edu> — *Regulatory networks governing nitrogen use efficiency in maize and sorghum*

P101 **Amanda Gilbert** <agilber@umn.edu> — *Structural and functional properties of core and dispensable genes in maize*

P102 **Taylor Strayhorn** <taylor.strayhorn@uga.edu> — *Systematic exploration of transcription factor function in maize*

P103 **Nikita Sajai** <ns623@cornell.edu> — *The association of indels with meiotic recombination sites in maize*

P104 **Jyothi Prasanth Durairaj Rajeswari** <jyothi@iastate.edu> — *The case for retaining natural language descriptions of phenotypes in plant databases and a web application as proof of concept*

P105 **Sohyun Bang** <Sohyun.Bang@uga.edu> — *Understanding the role of ZmWUS1 and cis-regulatory elements in maize inflorescence development at single-cell resolution*

P106 **Sidney Sitar** <sitarsid@msu.edu> — *Utilizing FT-MIR spectroscopy and LC-MS to model phenolic compound accumulation in diverse maize kernels*

P107 **Jingjing Zhai** <jz963@cornell.edu> — *reelProtein: filtering functional protein annotations with machine learning*

Cytogenetics

P108 **Mateus Mondin** <mmondin@usp.br> — *A new B chromosome repeat and its relationship to other repetitive sequences of maize*

P109 **Hafiza Sara Akram** <ha20be@fsu.edu> — *Cytological evidence supports the two-compartment model of maize euchromatin distinguished by replication timing*

P110 **James Birchler** <birchlerj@missouri.edu> — *Genomic conflict between the A and B chromosomes in High Loss lines*

P111 **Mateus Mondin** <mmondin@usp.br> — *Heterochromatic knobs exhibit differences in chromatin accessibility during maize development*

P112 **Mingyu Wang** <mw36149@uga.edu> — *Kinesin-10-like is a candidate adaptor protein that mediates the interaction between KINDR and knob180 repeats, facilitating meiotic drive of abnormal chromosome 10*

P113 **Yibing Zeng** <yz77862@uga.edu> — *Stability of chromosome segregation by synthetic maize centromeres*

Education & Outreach

P114 **Aimee Schulz** <ajs692@cornell.edu> — *A Seed Dispersal Game: curriculum for teaching plant domestication and adaptation to students of all ages*

P115 **Gibum Yi** <gibumyi@gmail.com> — *DNA extraction method from a seed without damaging to germination ability in maize (Zea mays L.)*

P116 **Stephen Gray** <sjgray4@iastate.edu> — *Documenting successful applications of plant genetic resources*

P117 **Gregory Schoenbaum** <gregorys@iastate.edu> — *Effects of plot orientation and leaf angle on maize grain yield*

P118 **Brandi Sigmon** <bsigmon2@unl.edu> — *Encouraging early pursuit of experiential learning in STEM education*

P119 **Removed** — *Abstract has been removed from the program*

P120 **Vivian Bernau** <vivian.bernau@usda.gov> — *Managing and distributing maize diversity: The NCRPIS maize collection*

P121 **Irina Makarevitch** <imakarevitch01@hamline.edu> — *The Maize Building Better Together Workshop: Strategies for a healthy and inclusive research community*

P122 **Bianca Sheridan** <bsheridan@fsu.edu> — *The Maize-10-Maze project, an educational public chromosome map garden featuring the mutants of maize.*

P123 **Vivian Bernau** <vivian.bernau@usda.gov> — *The Wilkes Legacy Collection: The modern era of teosinte research began here*

Quantitative Genetics & Breeding

P124 **Aurelien Beugnot** <aurelien.beugnot@gmail.com> — *A LD decay method for determining confidence intervals of QTL in GWAS and perform QTL clustering*

P125 **Alexander Liu** <aliu@danforthcenter.org> — *A Rootless1 knockdown allele affects maize nodal root development, changing root system architecture and function.*

P126 **Sylvie Coursol** <sylvie.coursol@inrae.fr> — *A combination of quantitative trait locus mapping and transcriptome analysis reveals a cluster of three MYC2- and jasmonic acid-dependent genes associated with chilling tolerance in maize*

P127 **Sumeet Mankar** <sumeetmankar171@gmail.com> — *A comprehensive phenomics study of above and below-ground traits in a maize diversity panel under nitrogen limitation*

P128 **Peter Balint-Kurti** <pjbalint@ncsu.edu> — *A leucine rich repeat receptor kinase gene confers quantitative susceptibility to maize southern leaf blight*

P129 **Zachary Traylor** <zbtyxb@umsystem.edu> — *A new ethanol extraction method in maize using lab-scale, dry-grind technology*

P130 **Robert Shrote** <shrotero@msu.edu> — *A novel multi-objective genomic selection strategy improves long-term genetic gains through improved diversity maintenance*

P131 **Dylan Schoemaker** <schoemaker@wisc.edu> — *A practical and cost-effective method to improve the efficiency of seed production for genetics research and breeding operations*

P132 **Matthew Runyon** <mrunyon2@illinois.edu> — *A single-gene reduction in leaf area demonstrates potential for improved planting density tolerance in elite maize hybrids*

P133 **Jaclyn Nicole Uy** <uyjnr69@hawaii.edu> — *Adopting modern maize transformation technologies for sustainable crop improvement in Hawaii*

P134 **Tae-Chun Park** <tcpark@iastate.edu> — *Application of DH technology and molecular markers to combine multiple major genes for improving corn quality*

P135 **Germano Costa-Neto** <gmcn222@gmail.com> — *Association mapping for environmental-dependent alleles in maize is leveraged by climate and soil kinships*

P136 **Steve Moose** <smoose@illinois.edu> — *Breeding BeerCorn, maize hybrids with enhanced performance for brewing beer*

P137 **Jonathan Niyorukundo** <niyorukundoj@gmail.com> — *Breeding for color and nutritional content in sweet corn and popcorn*

P138 **Young Sam Go** <ysgo@korea.kr> — *Breeding of maize double recessive mutant lines using selection markers*

P139 **Timothy Kelliher** <Tim.Kelliher@syngenta.com> — *Breeding transformable haploid inducers for elite line genome editing*

P140 **Joseph DeTemple** <detemplj@iastate.edu> — *CERIS-JGRA analysis of flowering time in CML277 and Tzi8*

P141 **Alex Mullens** <mullens3@illinois.edu> — *Characterization and mapping of vascular and nonvascular colonization of maize by Xanthomonas vasicola pv. vasculorum*

P142 **Alison Uberti** <auberti@iastate.edu> — *Combining heterotic groups for maize root seedling traits increases the power of genomic prediction accuracy under low phosphorus*

P143 **Alice Silva Santana** <asantana@iastate.edu> — *Comparison of genomic selection models for maize seedling traits under contrasting phosphorus conditions*

P144 **Kiara Kappelmann** <kiarak@iastate.edu> — *Comprehensive kernel analysis in large DH populations derived from intercrossing germplasm enhancement of maize lines*

P145 **Lauren Whitt** <lwhitt@danforthcenter.org> — *Computational methods to identify ionome genes*

P146 **Sylvia Morais de Sousa Tinoco** <sylvia.sousa@embrapa.br> — *Crop type determines the relation between root system architecture and microbial diversity indices in different phosphate fertilization conditions*

P147 **Karlene Negus** <knegus@iastate.edu> — *Current applications of neural networks for genomic prediction*

P148 **Brooke Bouwens** <brookebouwens317@gmail.com> — *Determining hybrid status using maize leaf reflectance and quantifying spectral plasticity in response to nitrogen treatmentspectral plasticity in response to nitrogen treatment*

P149 **Madison Mitchell** <mnmzbd@umsystem.edu> — *Drone-based identification of flood-tolerant maize genotypes*

P150 **Nikee Shrestha** <nshrestha5@huskers.unl.edu> — *Employing environmental indices to augment cross-environment prediction accuracy in diverse maize populations*

P151 **Bryan Panek** <bpp122@psu.edu> — *Evaluation of hybrid maize expressing flavonoids for fall armyworm control*

P152 **Ravi Mural** <rmural2@unl.edu> — *Exploring the genetic determinants of phenotypic means and plasticity for flowering time in a Maize Association Panel*

P153 **Tyler Foster** <tfoster@iastate.edu> — *Fine-mapping efforts reduced chromosomal region responsible for qshgd1 in maize*

P154 **Sarah Oliver** <sloxmd@umsystem.edu> — *Free asparagine in staple crops and its affinity to form acrylamide*

P155 **Destiny Tyson** <dntyson@ncsu.edu> — *Genetic characterization of a potentially new fertilization barrier system with unlinked male and female controls*

P156 **Raksha Singh** <raksha.singh@usda.gov> — *Genetic dissection using nested-association mapping (NAM) reveals quantitative trait loci (QTL) conferring resistance to Tar Spot in maize.*

P157 **Boris M. E. Alladassi** <aboris@iastate.edu> — *Genetics of leaf angle across canopy levels in maize*

P158 **Joseph Atemia** <j.atemia@fz-juelich.de> — *Genome-wide association studies to establish the genetic basis of agro-mophological and climatic traits in wild maize relatives*

P159 **Jacob Hinrichsen** <jhinrich@iastate.edu> — *Genome-wide dissection of leaf angle variation across the canopy in maize*

P160 **Alizarine Lorenzi** <alizarine.lorenzi@inrae.fr> — *Genomic prediction across two breeding cycles in maize silage using a factorial approach*

P161 **Jenifer Camila Godoy dos Santos** <godoycamilajds@usp.br>
Genomic selection models for untested maize hybrids performance prediction in non-evaluated environments

P162 **Stephanie Klein** <spklein@iastate.edu>
Genotype-specific differences in the transcriptional landscape underlie varying root phenotypes under nitrogen stress

P163 **Kyle King** <kylek@iastate.edu>
Grain yield genetic gain for Bayer Crop Science maize hybrids

P164 **Alejo Ruiz** <aruiz@iastate.edu>
Harvest index has increased over the last 50 years of maize breeding

P165 **Melissa Draves** <madcfr@umsystem.edu>
Heirloom maize of the United States and Canada

P166 **Piyush Pandey** <piyush.pandey@usda.gov>
High-throughput mutant screening using unoccupied aerial systems

P167 **Jensina Davis** <jdavis132@huskers.unl.edu>
High-throughput phenotyping of phyllotaxy via 3D reconstruction enables quantitative genetic analysis of canopy architecture

P168 **Maxime Laurent** <maxime.laurent@uclouvain.be>
Identification and characterization of transcription factors regulating aquaporin gene expression

P169 **Kirsten Hein** <kirsten.hein@colostate.edu>
Identification of drought-adaptive QTL underlying variation in root system architecture in Zea mays

P170 **Dayane Cristina Lima** <dclima@wisc.edu>
Identification of drought-tolerant hybrids originating from elite parents

P171 **Mercy Fakude** <mfakude@iastate.edu>
Identification of genomic region/s associated with the causal QTL of SHDG trait in Ames panel by GWAS

P172 **Alberto Tassinari** <alberto.tassinari8@unibo.it>
Identification of novel QTLs for ear fasciation, kernel row number, ear prolificacy and tillering in maize (Zea mays L.)

P173 **Grace Sidberry** <gcsfgv@umsystem.edu>
Identifying drought tolerance loci through automated root phenotyping and GWAS

P174 **Eric Rdoene** <eric.rodene@huskers.unl.edu>
Image filtering to improve maize tassel detection accuracy using machine learning algorithms

P175 **Yawei Li** <yaweil@iastate.edu>
Image-based high-throughput phenotyping and genetic analysis of plant architecture in maize

P176 **Alain Charcosset** <alain.charcosset@inrae.fr>
Improving the use of plant genetic resources to sustain breeding programs efficiency

P177 **Liz Dominguez** <lizethd2@illinois.edu>
Increased stem sink strength and sugar accumulation using male sterility in Sorghum bicolor

P178 **Asher Hudson** <ahudson3@ncsu.edu>
Investigating heterosis for resistance to three pathogens

P179 **Robert Twohey III** <twohey2@illinois.edu>
Investigating leaf-water relations and stomatal control in Zea mays using the mutant slac1-2

P180 **Matthew Wendt** <wendtmat@iastate.edu>
Key developmental windows and environmental parameters that influence cuticular wax composition on maize silks

P181 **Haixiao Hu** <hxhu@ucdavis.edu>
Large-scale multi-environment genomic prediction for maize hybrid selection

P182 **Rafael Della Coletta** <della028@umn.edu>
Linking genetic and environmental factors through marker effect networks to understand trait plasticity

P183 **Ella Townsend** <ellatown@iastate.edu>
Low-cost phenotyping of seedling root growth angle shows genotypic variation and plastic response to parental nitrogen input

P184 **Sotirios Archontoulis** <sarchont@iastate.edu>
Maize breeding impacts on sustainability

P185 **Daniel Laspisa** <Daniel.Laspisa@uga.edu> — *Mapping maize brace root traits associated with nitrogen-fixing bacterial symbiosis*

P186 **Norbert Bokros** <nbo245@uky.edu> — *Metabolic pathway associations of maize stalk lodging resistance*

P187 **Angela Kent** <akent@illinois.edu> — *Mining ancient genomes for crops of the future*

P188 **Grace Nystrom** <gln2@illinois.edu> — *Monitoring nitrogen-responsive phenotypes in corn with the TerraSentia Rover*

P189 **Ty Thomas** <tsthoma2@ncsu.edu> — *Multi-environmental RNAseq reveals the extent of Genotype-by-Environment interactions for gene expression.*

P190 **Collin Luebbert** <cluebbert@danforthcenter.org> — *Multi-year, holistic phenotyping reveals genetic diversity in nitrogen related traits*

P191 **Diana Marcela Escamilla Sanchez** <descamil@iastate.edu> — *Optimizing genomic prediction for the germplasm enhancement of maize project*

P192 **Linda Dao** <ldao@ufl.edu> — *Pericarp thickness in sweet corn inbreds and hybrids*

P193 **Julian Cooper** <coop0409@umn.edu> — *Phenomic and genomic temporal analysis of maize canopy cover*

P194 **Jianming Yu** <jmyu@iastate.edu> — *Phenotypic Plasticity: A broad framework to reinstate the environmental dimension for GWAS and genomic selection*

P195 **Hongyu Jin** <hjin5@huskers.unl.edu> — *Phenotypic characterization of sorghum nitrogen responsive gene edits using high- throughput and conventional phenotyping throughput and conventional phenotyping*

P196 **Kyle Linders** <klinders@huskers.unl.edu> — *Plasticity of sorghum biomass and inflorescence traits in response to nitrogen application*

P197 **Danielle Davis** <dtdqkd@umsystems.edu> — *Protein and starch variation in heirloom corn varieties and their properties in food.*

P198 **Michael Burns** <burns756@umn.edu> — *Quantification of pericarp retention of nixtamalized corn using image analysis*

P199 **Dongdong Li** <ddli@iastate.edu> — *Quantitative genetics in the pangenome era*

P200 **Lia Olmedo Pico** <lolmedop@purdue.edu> — *Relative dependency of grain yield and quality changes of maize hybrids released from 1980 to 2020 on nitrogen availability*

P201 **Sendi Mejia** <smejiaji@purdue.edu> — *Screening a highly efficacious mutagenized maize library for mutants that are more susceptible or resistant to P.maydis*

P202 **Eric Butoto** <ebutoto@ncsu.edu> — *Selection for early flowering in five tropical maize populations with varying initial genetic diversity*

P203 **Removed** — *Abstract has been removed.*

P204 **Forrest Li** <frrli@ucdavis.edu> — *Single-gene resolution of locally adaptive genetic variation in Mexican maize*

P205 **Joan Barreto Ortiz** <jbarreto@umn.edu> — *SpykProps: An imaging pipeline to quantify the spike architecture in perennial ryegrass*

P206 **Bharath Kunduru** <bkundur@clemson.edu> — *Standardization of phenotyping and analytical approaches to assess stalk lodging resistance in maize*

P207 **Yu-RU Chen** <yuruchen@iastate.edu> — *Superior haploid inducers are attained from doubled inducer haploids*

P208 **Dorothy Sweet** <kirsc168@umn.edu> — *Temporally resolved growth patterns reveal variability in environmental responsiveness in diverse maize panel*

P209 **Merritt Khaipho-Burch** <mbb262@cornell.edu>
The perils and promise of single-gene solutions to crop yield: extraordinary claims require extraordinary evidence

P210 **Gen Xu** <gxu6@unl.edu>
Trans-eQTL contributes to gene expression heterosis in maize

P211 **Zhongjie Ji** <jizhongj@msu.edu>
Undertaker: a stereo imaging system to explore latent canopy traits

P212 **Nate Korth** <nkorth@huskers.unl.edu>
Using the human gut microbiome as a phenotype of sorghum in a genetic mapping study

P213 **Sophia Schmidt** <sophie7@iastate.edu>
Using variance component analysis to connect single parent expression to phenotype

P214 **Patrick Woods** <patrick.woods@colostate.edu>
Validation of a root system architecture QTL and insight into the impact of structural variants on GWAS in maize

P215 **Michelle Cho** <michelle.s.cho@wustl.edu>
ZmIRA1, a new mediator between Root System Architecture (RSA) and nitrogen metabolism

P216 **Alain Charcosset** <alain.charcosset@inrae.fr>
metaGE: Investigating genotype x environment interactions through GWAS meta-analysis

Transposons & Epigenetics

P217 **Kevin Peek** <k.peek@uva.nl>
A role for RdDM in susceptibility to paramutation at the maize BOOSTER1 locus

P218 **Removed**
Poster removed at the request of the author

P219 **Bimala Acharya** <bacharya@iastate.edu>
Harnessing the helitron

P220 **William Clore** <whclore@iastate.edu>
LTR Predictor: A tool to identify LTR retrotransposon insertions in long-read genome sequencing data

P221 **Merritt Khaipho-Burch** <mbb262@cornell.edu>
Limited contribution of transposable elements to regulatory adaptation in maize inbreds and hybrids

P222 **Jonathan Cahn** <cahnjonathan@gmail.com>
Maizecode: DNA regulatory elements in maize and teosinte inbreds provide insight into maize domestication

P223 **Kaitlin Higgins** <kaitlinj@iastate.edu>
Mdr1 demethylase and the intersection between the epigenome, genomic imprinting, and transposable elements in maize endosperm

P224 **Nathan Catlin** <catlinna@msu.edu>
Methods for detecting TE PAVs in a maize diversity panel

P225 **Jonathan Gent** <gent@uga.edu>
Natural methylation epialleles correlate with gene expression in maize

P226 **Ruth Epstein** <rke27@cornell.edu>
Predicting the fine scale crossover landscape in maize

P227 **Nadia Mourad Silva** <nmourad@ufl.edu>
See what's new at UniformMu

P228 **Maike Stam** <m.e.stam@uva.nl>
Silencing release at b1 gene in RdDM mutants is associated with reduced H3K9me2 and H3K27me2 rather than DNA methylation

P229 **Claire Menard** <menar060@umn.edu>
TIPs and tricks for identifying transposable element insertion polymorphisms in large genomes at the population-level

P230 **Peng Liu** <mcliupeng@gmail.com>
Targeted transposition in Arabidopsis

P231 **Andrew Read** <andycread@gmail.com>
The impact of genic structural variants on gene expression profiles across the NAM founder lines

P232 **Jason Lynn** <jlynn@cshl.edu> — *Towards creating a comprehensive ARGONAUTE mutant collection in maize using CRISPR-Cas9*

P233 **Jeff Bennetzen** <maize@uga.edu> — *Transposable elements as actors and sensors in the genome dynamics of maize and other flowering plants*

Evolution and Population Genetics

P234 **Sierra Raglin** <sraglin2@illinois.edu> — *Blast from the Past: Maize breeding history and the rhizosphere microbiome*

P235 **Charlie Hale** <coh22@cornell.edu> — *Characterizing cis-regulatory evolution at scale across hundreds of wild grass species*

P236 **Nirwan Tandukar** <ntanduk@ncsu.edu> — *Convergent adaptation of maize and sorghum to soils with low phosphorus availability.*

P237 **Meghan Brady** <meghan.brady@uga.edu> — *Detection of abnormal chromosome 10 in genotype-by-sequencing data*

P238 **Madsen Sullivan** <madsens2@illinois.edu> — *Genetic characterization and population structure of North American popcorn*

P239 **Catherine Li** <chli6@illinois.edu> — *Genetic dissection of kernel protein concentration using near-isogenic lines derived from the Illinois long term selection experiment containing the FLOURY2-RFP reporter*

P240 **Heather Chamberlain-Irwin** <hchamber@iastate.edu> — *Genetic legacies of the first ancient South American state in archaeological maize*

P241 **Arun Seetharam** <arnstrm@iastate.edu> — *Genomes of 35 representative species of Andropogoneae Clade provide insight into the evolution, diversification, and adaptation of major crops*

P242 **Elad Oren** <eo235@cornell.edu> — *How does a maize relative withstand deep freezing temperatures?*

P243 **Samantha Snodgrass** <snodgras@iastate.edu> — *Identifying fractionation events across the Tripsacinae subtribe*

P244 **Elena Jiang-Grieser** <elenaj@iastate.edu> — *Identifying structural variation across diverse Zea genomes*

P245 **Mohamed El-Walid** <mze3@cornell.edu> — *Mapping freezing tolerance in tripsacum by bulk segregant analysis*

P246 **Elli Cryan** <epcryan@ucdavis.edu> — *Molecular evolution of a reproductive barrier across twelve million years*

P247 **Sheng-Kai Hsu** <sh2246@cornell.edu> — *Phylogenetic variation of potential nitrification rate in rhizosphere of diverse grass species in Andropogoneae*

P248 **Michael Anokye** <anomic17@gmail.com> — *The Hordeum genus: A rich Resource for adaptive genetic variation*

P249 **Amruta Bapat** <amruta03@iastate.edu> — *The Ga1 locus of the genus Zea is associated with genome structures derived from multiple, independent non-homologous recombination events*

Biochemical and Molecular Genetics

P250 **Chris Larson** <christopher.d.larson@und.edu> — *A Mendelian genetic model to explain the non-Mendelian segregation ratios of UND-9*

P251 **Emily Kuhn** <kuhn43@purdue.edu> — *A Novel mutational approach to uncover genetic determinants of hybrid vigor in maize*

P252 **Janik Telleria Marloth** <janik.telleria-marloth@pharmazie.uni-halle.de> — *A maize protoplast assay provides insight into the regulation of terpene synthase 23, a gene involved in plant defense against Western Corn Rootworm*

P253 **Dirk Winkelman** <dwink@iastate.edu> — *A multidisciplinary approach to assess the roles of Glossy2 and Glossy2-like in maize cuticular wax biosynthesis*

P254 **Singha Dhungana** <srdm93@umsystem.edu> — *A novel DNAJ-thioredoxin-like protein impacts carbohydrate partitioning in maize*

P255 **Eric Schmelz** <eschmelz@ucsd.edu> — *A shared maize biochemical defense pathway enzyme contributes to plant growth*

P256 **Hwan-Hee Bae** <miami9@korea.kr> — *Analysis of phytochemical accumulation and antioxidant activity during maturation of corn grains containing both carotenoids and anthocyanins*

P257 **Matthias Langer** <langer@ipk-gatersleben.de> — *Causes and consequences of endogenous hypoxia on growth and metabolism of the developing maize kernel*

P258 **Madison Lane** <mlane@iastate.edu> — *Characterization of candidate genes related to cuticular wax deposition on maize silks*

P259 **Jason Roberts** <jtrgmc@umsystem.edu> — *Characterization of seedling growth and embryonic root systems of carbohydrate partitioning defective mutants in maize*

P260 **Nan Jiang** <jiangn11@msu.edu> — *Combinatorial effects of the ACT-like domain and small molecule on the regulatory activity of the maize bHLH transcription factor R1*

P261 **Leah Durst** <durstlea@msu.edu> — *Comparing phenolic compound accumulation in maize and sorghum*

P262 **Lauren Higa** <higalaur@hawaii.edu> — *Developing an efficient gene-editing method for tropical maize protoplasts using CRISPR/Cas9*

P263 **Huda Ansaf** <hah34@umsystem.edu> — *Development of multi-functional CRISPR system in fast-flowering mini maize to study proteomic rebalancing and other complex metabolic traits*

P264 **Hope Hersh** <hopehersh@ufl.edu> — *Engineered 6-phosphogluconate dehydrogenase in amyloplasts assessed in field corn hybrids for mitigation of grain yield loss under heat stress*

P265 **Ruthie Stokes** <rstokes@ncsu.edu> — *Fine tune regulation of flowering time in maize via phospholipid interaction with ZCN8*

P266 **Hannah Pil** <hdpil@ncsu.edu> — *Functional analysis of High PhosphatidylCholine 1 (hpc1): A phospholipase involved in maize adaptation to high elevations*

P267 **Elysse Trost** <eltrost@iastate.edu> — *Functional characterization of genes in the maize fatty acid elongation pathway using synthetic biology approaches in yeast*

P268 **Lina Gomez Cano** <gomezca5@msu.edu> — *GWAS identification of genes associated with phenolic metabolism*

P269 **Maruti Nandan Rai** <mnrai@illinois.edu> — *Genetic variation in NRT1.1b contributes to improved nitrogen utilization in maize*

P270 **Namrata Jaiswal** <namrata.jaiswal@usda.gov> — *Heterologous expression in Nicotiana benthamiana identifies candidate effector proteins from Phyllachora maydis that suppress cell surface-triggered immune responses*

P271 **Kong Wong** <kwong@danforthcenter.org> — *In situ quantification of plant carbon allocation in a Maize-AMF system*

P272 **Sylvia Morais de Sousa Tinoco** <sylvia.sousa@embrapa.br> — *Inoculation with selected strains of Bacillus megaterium and B. subtilis, present in the first inoculant released in Brazil, increases maize phosphorus acquisition and yield*

P273 **Tyler Lesko** <tkl5215@psu.edu>
Insecticidal 3-deoxyanthocyanidin flavonoids from maize and sorghum to fanage fall armyworm

P274 **Charles Hunter** <cthunter3@gmail.com>
Loss of Allene Oxide Cyclase results in developmental changes and hypersensitivity to fungal and insect pests due to jasmonic acid deficiency

P275 **Fangyi Li** <fangyihuskers@gmail.com>
Low-cost photosynthetic fluorescence phenotypic solutions for GWAS

P276 **Tianxiao Yang** <tianxiao.yang@ufl.edu>
Maize rough endosperm6 (rgh6) is predicted to affect RNA processing in endosperm development

P277 **Cassandra Eddy** <cassie.eddy01@gmail.com>
Maize transcriptional and microRNA regulation by gibberellic acid

P278 **Saet-Byul Kim** <saetbyul.kim@unl.edu>
Molecular characterization of a common rust effector AvrRp1-D recognized by maize immune receptor Rp1-D

P279 **Tyler Ferris** <tyler.ferris@huskers.unl.edu>
Nutritional and biophysical characteristics of CRISPR generated low kafirin, waxy sorghum

P280 **Rohit Kumar** <mohank@clemson.edu>
Proteomic and metabolomic landscape of association between maize root and arbuscular mycorrhizal fungi

P281 **Mae Antonette Mercado** <mercado6@illinois.edu>
RUBISCO activase3 (rca3) and its potential role in heat stress response in maize

P282 **Brianna Griffin** <bdg@iastate.edu>
Roles of REL2 mediated transcriptional co-repression in maize immunity

P283 **Nathalie Walter** <nwalter8888@gmail.com>
Seed expressed scorable markers facilitating seed sorting to accelerate plant biology research

P284 **Rajdeep Khangura** <rkhangur@purdue.edu>
Semi-dominant maize mutant Bella fleck1 provides resistance to multiple fungal diseases

P285 **Nadia Mourad Silva** <nmourad@ufl.edu>
Small-kernel ears result when Sorbitol dehydrogenase1 (Sdh1) is dysfunctional

P286 **Brian Dilkes** <bdilkes@purdue.edu>
Surprising non-linear phenotypic impacts of variation in chlorophyll affected by natural and induced variation the Mg2+ Chelatase subunit I

P287 **Jiahn-Chou Guan** <guanjc@ufl.edu>
Targeting changes in strigolactone biosynthesis to confer witchweed resistance without yield penalty in maize

P288 **Ray Collier** <rcollier2@wisc.edu>
The Wisconsin Crop Innovation Center: a public resource for maize transformation and editing service, research and discovery

P289 **Larissa Barl** <larissa.barl@tum.de>
The potential of the ABA-Hydroxylase gene family for fine-tuning of water use efficiency and drought-related traits in maize

P290 **Manwinder Singh Brar** <mbrar@clemson.edu>
Time-course metabolome variation in genetically diverse inbred lines identify pathways underlying staygreen trait in maize

P291 **Samuel Herr** <skh77@cornell.edu>
Uncovering the link between chlorophyll synthesis and vitamin E in maize grain

P292 **Ran Tian** <rtian@ttu.edu>
Understanding the molecular and evolutionary mechanisms of epicuticular wax in sorghum

P293 **Ha Duong** <hndvw5@umsystem.edu>
Understanding the genetic architecture controlling seed amino acid composition in arabidopsis

P294 **Betina Debastiani Benato** <betina.benato@tum.de>
Unravelling the relationship between stomatal properties and Cercospora zeae-maydis infection in maize

P295 **Amanpreet Kaur** <kaur60@purdue.edu>
Using gene expression patterns as signatures of a biological response reveals the rheostat-like nature of brassinosteroid signaling

Late Posters

P296 **Mirai Maeda Inaoka**
<minaoka2@illinois.edu>

Genetic markers associated with leaf angle and tassel branch number in maize

Plenary Speaker Abstracts

Invited Speaker 1 **Friday, March 17 10:50 AM CDT**

Genetic engineering of chloroplast and mitochondrial genomes

(submitted by Ralph Bock <rbock@mpimp-golm.mpg.de>)
Full Author List: Bock, Ralph[1]
[1] Max Planck Institute of Molecular Plant Physiology, Am Mühlenberg 1, D-14476 Potsdam-Golm, Germany

The genomes of plastids (chloroplasts) and mitochondria represent attractive targets of genetic engineering in crop plants. Plastid transgenes often give very high expression levels, can be conveniently stacked in synthetic operons, and are largely excluded from pollen transmission, thus providing increased biosafety of transgenic crops. Recent research has greatly expanded our toolbox for plastid genome engineering, and a number of proof-of-principle applications have highlighted the enormous potential of the transplastomic technology in crop improvement and for the use of plants as bioreactors for the sustainable and cost-effective production of biopharmaceuticals, enzymes and raw materials for the chemical industry. While a technology for transgene insertion into mitochondrial genomes is not yet available, the recent development of genome editing methods for mitochondria has enabled the targeted manipulation of endogenous mitochondrial genes. In my talk, I will (i) describe the state of the art in chloroplast genome engineering, (ii) highlight selected applications of plastid transformation in biotechnology and synthetic biology, and (iii) discuss recent advances with engineering the mitochondrial genomes of plants.

Funding acknowledgement: Max Planck Society

Invited Speaker 2 **Friday, March 17 11:40 AM CDT**

From genome to phenome: Understanding the diversity of maize

(submitted by Candice Hirsch <cnhirsch@umn.edu>)
Full Author List: Hirsch, Candice[1]
[1] University of Minnesota, St Paul, MN, 55108

Maize is an extraordinarily diverse species. At the genome level we have demonstrated extensive structural variation in the maize pan-genome beginning with early pan-transcriptomics studies. Recent advances in sequencing technologies and assembly algorithms have allowed us, for the first time, to characterize this variation across the entirety of the genome. Pacbio-based assemblies of the 26 NAM founder lines are allowing comparative genomic analyses typically conducted across species to be applied to characterize the extensive variation within maize with regards to both genes and transposable elements. This variation in genome content is able to explain phenotypic variation in the maize NAM population that is not captured by SNP variation alone and to provide insights into genome variation that has facilitated local adaptation of the species. We have also shown these structural variants drive dynamic gene expression patterns in inbreds and expression complementation in hybrids. This variation in the maize pan-genome also drives the variation in plasticity and environmental responsiveness that is observed within the species. This talk will discuss our current understanding of variation in the maize pan-genome and the extensive variation in drives in phenotypic diversity and plasticity.

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

Understanding mechanisms of thermoadaptation of desert extremophiles

(submitted by Seung Yon (Sue) Rhee <srhee@carnegiescience.edu>)
Full Author List: Rhee, Seung Yon (Sue)[1]
[1] Carnegie Institution for Science

Global warming is changing the habitability of many places for many species on Earth. Plants occupy the largest portion of the land surface and understanding thermoadaptation in plants is critical and timely for global sustainability, food security, and species conservation. Thermophiles have adapted to thrive under extreme heat, but little is known about thermoadaptive mechanisms in thermophilic plants. *Tidestromia oblongifolia* is a flowering plant belonging to the Amaranthaceae family. Endemic to the hot deserts of the US Southwest, *T. oblongifolia* has an optimal photosynthetic rate at 47ºC and can increase its biomass by up to 30% daily in Death Valley summers. To understand how this plant can thrive at such high temperatures, we recreated Death Valley summer conditions in custom-engineered high-temperature growth cabinets, obtained seeds of two accessions of *T. oblongifolia* (Death Valley and Dos Palmas), sequenced their genomes, and examined their physiology, cellular and organellar structures, transcriptomics, and metabolomics in response to high temperature and high light conditions that mimic Death Valley summers. I will describe our findings in this talk. We anticipate that this study may provide new insights into the thermal limit of photosynthesis in flowering plants, which could improve thermoadaptation in crops, develop industrial biocatalysts, and address conservation of plants in the context of climate change.

Invited Speaker 4 **Saturday, March 18 11:40 AM CDT**

Local and global perspectives on the causes and consequences of epigenetic silencing of transposable elements

(submitted by Damon Lisch <dlisch@purdue.edu>)
Full Author List: Lisch, Damon[1]
[1] Purdue University; West Lafayette, Indiana, USA 47907

The maize genome has a lot of transposable elements (TEs). Indeed, it is mostly TEs. Many, probably the vast majority, are broken in some way and cannot replicate. If there is junk in the genome, these are that. Likely because these elements can serve as templates for ectopic recombination, these broken elements are kept in a deeply silenced heterochromatic state. However, some TEs remain active, and some are highly mutagenic. It is these elements that that must be recognized by the host and silenced *de novo*, resulting in new and highly polymorphic islands of silenced chromatin. This is the case for *MuDR* elements, whose silencing can be triggered by a trans-acting variant of *MuDR* called *Muk*. Remarkably, using only this system, we have found evidence of nearly every kind of epigenetic silencing known in plants, consistent with ancient and ongoing roles for all of these pathways in TE control. In addition, we have evidence that despite its moniker, the *Mutator*system is a remarkably bad mutagen. Although these elements target the 5'UTR of genes, they frequently have little effect on gene expression. However, over longer periods of time these and other silenced insertions are purged. This has led to the suggestion that silencing of TEs near genes represents a compromise between maintaining genome integrity and optimal levels of gene expression. A consequence of this appears to be subgenome dominance in ancient polyploids like maize and soybean, each of which has followed its own distinct evolutionary trajectory, but both of which appear to have been profoundly influenced by TEs. Together, these observations, from the anecdotal to the general, the local to the global, suggest that the fate of TEs and their hosts are inextricably joined, and that what we learn from one can inform our understanding of the other.

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

Invited Speaker 5 **Saturday, March 18 8:40 PM CDT**

Sowing the seeds of equity to reap a harvest of diverse scientists

(submitted by MariaElena Zavala <mariaelena.zavala@csun.edu>)
Full Author List: Zavala, MariaElena[1]
[1] Department of Biology, California State University Northridge, Northridge CA

People are naturally curious about the world around them. However, not all people are encouraged to follow their interests or to satisfy their curiosity by actively engaging in science. Why is that so? What is the genesis of perception that only a chosen few can or should become scientists? How must we prepare the science soil so that students will have access to opportunities that will encourage their curiosity and support their desire to participate in the great adventure of scientific discovery? Using data from programs that I proposed and implemented at CSUN, we will show that these programs not only prepared students for careers in basic biomedical research but also stimulated changes in the culture of the university to be more inclusive and student focused.

McClintock Prize Abstract

McClintock Prize (M1) **Friday, March 17 8:10 PM CDT**

Development of maize anthers…it's a long road from lobe inception to functional pollen

(submitted by Virginia Walbot <walbot@stanford.edu>)
[1] Stanford University, Stanford CA 94305-5020 USA

Utilizing the rich resources of hundreds of maize male-sterile mutants, my lab collaborated with Zac Cande and his group to classify such mutants by defect and timing relative to the normal pace and pattern of anther cell division and expansion. Early on we cloned several key genes (transcription factors, *ameiotic1*, *mac1*) critical for successful anther development and functions of specific cell types and used microarrays to assess transcriptome changes in mutants of these genes. We overturned the classic lineage model to establish germinal and somatic cell fates in anthers, demonstrating instead that hypoxia triggers germinal cell differentiation and that these cells then secrete MAC1 to program neighbors to become somatic cells. We discovered the 21-nt and 24-nt anther-specific phasiRNAs with collaborators. Collaborating with Blake Meyers and his group we now know that these small RNAs are essential for anther development at normal growing temperatures but can be dispensable at non-optimal temperatures. Transcriptome profiling using whole anthers or laser-dissected cell types followed by spatial profiling with *in situ* hybridization has mercifully been superseded by single cell transcriptomics today, affording us many new insights into the developmental trajectories and functions of specific cell types. We continue to clone key genes, to analyze the phenotypic impact of mutants by quantitative microscopy plus analysis of RNA and protein composition, and to design experiments to evaluate new hypotheses and prove mechanisms.

Funding acknowledgement: National Science Foundation (NSF)

Short Talk Abstracts

SESSION 1 – THE GENES THAT MAKE MAIZE
Chair: Matthew Hufford Thursday, March 16. 7:00 PM – 9:00 PM CDT

T1

Identification of a trans-acting factor required for B chromosome nondisjunction-a component of its drive mechanism

(submitted by Hua Yang <yanghu@missouri.edu>)
Full Author List: Yang, Hua[1]; Liu, Hua[3]; Hou, Jie[2]; Chen, Chen[2]; Cheng, Jianlin[2]; Yang, Bing[3]; Birchler, James[1]
[1] Division of Biological Sciences, University of Missouri, Columbia, Missouri, USA 65211
[2] Department of Electrical Engineering and Computer Science, University of Missouri, Columbia, MO 65211
[3] Division of Plant Science & Technology, University of Missouri, Columbia, MO 65211

In 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 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 analyses located the trans factors on the B chromosome cytologically. Recently, a well-assembled B chromosome sequence allowed us to map one of the trans factors to 2.7 Mb of the distal euchromatic region that contains a total of 34 genes. Transcriptomic analysis of mature pollen on lines with and without B chromosomes showed expression in this tissue for three of the 34 genes. In the 1970's, Wayne Carlson recovered three EMS-induced mutants of TB-9Sb that had lost the property of nondisjunction. These were found to each have a mutation or a deletion for B chromosome gene Zm00044a000666. This gene is one of those expressed in mature pollen. An analysis of the three TB-9Sb mutants showed that they don't recombine with each other, indicating no off-target lesions in another trans factor for nondisjunction. Finally, a CRISPR-Cas9 mutagenesis produced three deletions within Zm00044a000666, which eliminated non-disjunction in the T0 progeny, confirming it as a trans factor. The predicted function of this gene is to bind substrates for ubiquitin-mediated proteolysis. Understanding the action of this B-encoded gene will aid in elucidating the basis of this unique example of genetic drive.

Gene / Gene Models described: Zm00044a000666

Funding acknowledgement: National Science Foundation (NSF)

T2

Distinct roles of plastidial and cytosolic arogenate pathways for phenylalanine and tyrosine biosynthesis in kernel and plant development

(submitted by Hui Liu <liu.hui@ufl.edu>)
Full Author List: Liu, Hui[1 2]; El-Azaz, Jorge[3]; Maeda, Hiroshi[3]; Yokoyama, Ryo[3]; Block, Anna[4]; Shin, Doosan[2]; Kim, Jeongim[1 2]; Basset, Gilles[1 2]; Wu, Shan[2]; McCarty, Donald[1 2]; Suzuki, Masaharu[2]
[1] Plant Molecular and Cellular Biology Program, University of Florida, Gainesville, FL, USA
[2] Horticultural Sciences Department, University of Florida, Gainesville, FL, USA
[3] Department of Botany, University of Wisconsin-Madison, Madison, WI, USA
[4] Chemistry Research Unit, Center for Medical, Agricultural and Veterinary Entomology, U.S. Department of Agriculture-Agricultural Research Service, Gainesville, FL, USA

Understanding mechanisms that determine organ size is a fundamental theme in plants. The ratio of embryo and endosperm size is an important determinant of grain composition. However, zygotic mechanisms that determine relative size of embryo and endosperm are little understood. Our genetic screen for maize embryo-endosperm size mutants has uncovered several genes that have key roles in the coordination of two seed organs. Here we present our progress on analysis of the *big embryo 6* (*bige6*) mutant. *Bige6* gene encodes a plastid-localized prephenate aminotransferase (PPA-AT) in the phenylalanine (Phe) and tyrosine (Tyr) biosynthetic pathway. The *bige6* mutant causes embryo enlargement at the expense of the endosperm. While Phe and Tyr are essential compounds for plant viability, homozygous *bige6* mutants develop small fertile plants. We attribute the non-lethal phenotype to a paralog, *Bige6-like*, that interestingly encodes a cytosolic instead of plastidial form of the enzyme. In contrast to maize, some plants including Arabidopsis and rice have a single PPA-AT gene. Intriguingly, RT-PCR analysis identified alternative transcripts encoding plastid and cytosolic AtPPA-AT forms suggesting that a cytosolic arogenate pathway is broadly conserved in plants. We generated null mutants of Arabidopsis *PPA-AT* by CRISPR/cas9 and found the *atppa-at* mutant causes female gametophyte defects and early seed lethality. Transgenes expressing plastidial forms of AtPPA-AT and BIGE6 were able to rescue the *atppa-at* mutant whereas transgenes expressing cytosolic forms failed to restore seed development. Our results support evidence that the plastid pathway is the predominant source of Phe and Tyr biosynthesis but also show that the cytosolic arogenate pathway contributes substantially to seed and plant development at least in maize. To account for the unexpected large embryo phenotype, we hypothesize that *bige6* disrupts a critical metabolic balance maintained by plastid and cytosolic pathways in the developing embryo and endosperm as well as in vegetative tissues.

Funding acknowledgement: National Science Foundation (NSF)

T3

Genome-wide association study identifies a link between boron homeostasis and *benzoxazinless3* in maize

(submitted by Michaela Matthes <mmatthes@uni-bonn.de>)
Full Author List: Shrestha, Vivek[1]; Chu, Liuyang[2]; Hochholdinger, Frank[2]; Angelovici, Ruthie[1]; McSteen, Paula[1]; Matthes, Michaela[1 2]
[1] Division of Biological Sciences, Bond Life Sciences Center, Interdisciplinary Plant Group, and Missouri Maize Center; University of Missouri; Columbia, Missouri, USA 65211-7310
[2] INRES-Crop Functional Genomics; University of Bonn; Bonn, NRW, Germany, 53113

The micronutrient boron is of high agronomical importance, since deficiencies of this nutrient are wide spread worldwide and drastically reduce the yield of many major crops, including maize. Despite this importance, knowledge about the molecular components influencing and regulating the homeostasis of this nutrient is scarce. The only identified and characterized genes thus far are boron transporter genes. Their expression is directly affected by boron levels in the soil and overexpression of these genes was used in engineering plants tolerant of low or high soil boron levels. Interestingly, the expression of many diverse genes varies depending on the soil boron level, suggesting the existence of additional molecular components influencing or regulating boron levels. In order to contribute to the molecular understanding of boron homeostasis in maize, we performed genome-wide association studies using boron concentration in the leaves subtending the first ear of the maize 282 Goodman-Buckler association panel. We identified *benzoxazinless3* (*bx3*), which is involved in the biosynthesis of the secondary defense compound DIMBOA, to be significantly associated with boron concentration variation. Phenotypic characterization of *bx3* mutants showed that boron concentration in the ear leaves of this mutant is higher compared to B73 control plants. In addition, the *bx3* mutants depicted a leaf tip necrosis phenotype, a phenotype which had been associated with boron toxicity previously. The severity of this phenotype in the *bx3* mutant correlates with varying boron levels, corroborating the connection between the leaf tip necrosis phenotype and altered boron levels in *bx3*. Our study therefore links a non-boron transporter gene with boron homeostasis in maize, which to our knowledge is an unprecedented finding. This finding suggests a previously unknown mechanistic link between boron and the benzoxazinoid pathway and provides a novel target for breeding crops adapted to low boron levels.

Gene / Gene Models described: *bx3*; GRMZM2G167549
Funding acknowledgement: United States Department of Agriculture (USDA), DFG

T4

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

(submitted by Siddique Aboobucker <siddique@iastate.edu>)
Full Author List: Aboobucker, Siddique I.[1]; Zhou, Liming[1]; Lübberstedt, Thomas[1]
[1] Department of Agronomy, Iowa State University, Ames, IA, 50011

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.

Funding acknowledgement: United States Department of Agriculture (USDA), Foundation for Food and Agriculture Research

T5

A genetic tradeoff for tolerance to moderate and severe heat stress in US hybrid maize

(submitted by Aaron Kusmec <amkusmec@iastate.edu>)
Full Author List: Kusmec, Aaron[1]; Attigala, Lakshmi[1 2]; Dai, Xiongtao[3 4]; Srinivasan, Srikant[5 6]; Yeh, Cheng-Ting[5]; Schnable, Patrick S.[5]
[1] Department of Agronomy, Iowa State University; Ames, IA, USA 50011
[2] Department of Environmental Health and Safety, Iowa State University; Ames, IA, USA 50011
[3] Department of Statistics, Iowa State University; Ames, IA, USA 50011
[4] Division of Biostatistics, University of California Berkeley; Berkeley, CA, USA 94720
[5] Plant Sciences Institute, Iowa State University; Ames, IA USA 50011
[6] School of Computing and Electrical Engineering, Indian Institute of Technology Mandi; Kamand, Himachal Pradesh, India 175005

Global climate change is increasing average temperatures and the frequencies of extreme high temperatures. Past studies have documented a strong negative effect of exposures to temperatures >30 °C on hybrid maize yields. However, these studies could not disentangle genetic adaptation via artificial selection from changes in agronomic practices. Because most of the earliest maize hybrids are no longer available, side-by-side comparisons with modern hybrids under current field conditions are generally impossible. Here, we report on the collection and curation of 81 years of public yield trial records covering 4,730 maize hybrids, which enabled us to model genetic variation for temperature responses among maize hybrids. We show that selection may have indirectly and inconsistently contributed to the genetic adaptation of maize to moderate heat stress over this time period while preserving genetic variance for continued adaptation. However, our results reveal the existence of a genetic tradeoff for tolerance to moderate and severe heat stress, leading to a decrease in tolerance to severe heat stress over the same time period. Both trends are particularly conspicuous since the mid-1970s. Such a tradeoff poses challenges to the continued adaptation of maize to warming climates due to a projected increase in the frequency of extreme heat events. Nevertheless, given recent advances in phenomics, enviromics, and physiological modeling, our results offer a degree of optimism for the capacity of plant breeders to adapt maize to warming climates, assuming appropriate levels of R&D investment.

Funding acknowledgement: National Science Foundation (NSF), United States Department of Agriculture (USDA), Department of Energy (DOE), USDA-NIFA, Iowa State University

T6

Analyzing the impact of breeding, soil variability and management practices on maize root system architecture using X-ray imaging

(submitted by August Thies <athies@danforthcenter.org>)
Full Author List: Thies, August C.[1 2]; Sciaressi, Cintia[3]; Olmedo Pico, Lia B.[4]; King, Kyle A.[3]; Trifunovic, Slobodan[5]; Eudy, Douglas[5]; Vyn, Tony J.[4]; Archontoulis, Sotirios V.[3]; Topp, Christopher N.[1]
[1] Donald Danforth Plant Science Center; St. Louis, MO 63132
[2] Division of Plant Science & Technology; University of Missouri; Columbia, MO 65211
[3] Department of Agronomy; Iowa State University; Ames, IA 50011
[4] Department of Agronomy; Purdue University; West Lafayette, IN 47907
[5] Bayer Crop Science; Chesterfield, MO, USA 63017

Root system architecture (RSA) is influenced by genetics, environments and agricultural management, thus understanding how these factors interact with each other is vital for root-based crop improvement. This study examined the impact of maize breeding, environmental variation and plant density on RSA through comparisons of root traits from a panel of era hybrids. Twelve maize hybrids were selected from across four decades (1985-2015) of the Bayer Crop Science breeding program and planted at both the historical density of the hybrid (20k, 25k or 30k/acre) and a modern density (35k/acre) at three different fields in Iowa, Indiana, and Illinois. To explore differences in RSA, in 2021 and 2022, we shovel-excavated 720 root crowns at stage R3, averaging 6 samples per era hybrid at each density and field site. We generated digital 3D reconstructions using X-ray computed tomography and used a custom computational pipeline. 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 planting density 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

T7 @vla_torres

Measurement of expression from a limited number of genes is sufficient to predict flowering time in maize

(submitted by Vladimir Torres <vladimir.torres@unl.edu>)
Full Author List: Torres-Rodríguez, J. Vladimir[1 2 3]; Sun, Guangchao[1 2 3 4]; Mural, Ravi V.[1 2 3]; Schnable, James C.[1 2 3]
[1] Quantitative Life Sciences Initiative, University of Nebraska-Lincoln, Lincoln, NE, USA
[2] Center for Plant Science Innovation, University of Nebraska-Lincoln, Lincoln, NE, USA
[3] Department of Agronomy and Horticulture, University of Nebraska-Lincoln, Lincoln, NE, USA
[4] Advanced Diagnostic Laboratory, Department of Quantitative Health Sciences, Mayo Clinic, Rochester, MN, USA

Flowering time plays a crucial role in determining regional adaptation, and mismatch between flowering time and environment can substantially impair yield. Different approaches can be used to predict flowering time before conducting large scale field evaluation and phenotyping. The more accurate prediction of a trait using genetic markers could be hindered due to all the intermediate steps connecting the trait and their genetic basics. The use of some intermediate steps as predictors could improve the accuracy of the model. We evaluated the utility of employing gene expression data from different organs at different growth stages to predict both anthesis and silking time across multiple environments. Random Forest models with gene expression data from two different organ systems (seedling roots and whole-seedling) were trained to predict days to anthesis and days to silking over five environments. Subsampling analysis determined that maximum prediction accuracy was attained with only 500 genes using whole-seedling data and 100 genes when using seeding root data. The accuracy of predictions obtained from expression data of small numbers of flowering time data exceeded the prediction accuracy obtained from conventional genomic prediction models trained using 400,000 SNP markers. Genes whose expression was rated as highly important in random forest models for root or shoot expression included several with known roles in determining flowering time, like MADS-transcription factor 69 and MADS-transcription factor 67. Here we demonstrate that expression data from even small numbers of genes, quantified in non-target tissues in environments different from where phenotypes are scored, can still meet or exceed the accuracy of genomic prediction from SNP markers.

Gene / Gene Models described: *Mads67, Mads69*; Zm00001eb327040, Zm00001eb143080

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

T8 @annusingh1206

Genetic analysis of leaf functional and eco-physiological traits for optimized photosynthesis in sorghum

(submitted by Anuradha Singh <singha57@msu.edu>)
Full Author List: Singh, Anuradha[1]; Newton, Linsey[1]; Schnable, James[2]; Thompson, Addie[1]
[1] Department of Plant, Soil & Microbial Sciences; Michigan State University; East Lansing, MI 48824
[2] Center for Plant Science Innovation and Department of Agronomy and Horticulture, University of Nebraska-Lincoln, Lincoln, NE 68588

Seasonal drought is expected to reduce soil water availability in many agricultural production areas globally, requiring a deeper understanding of how drought affects leaf functional and eco-physiological traits. Leaf developmental traits and stomatal features are the basic leaf functional traits that facilitate CO2 uptake for photosynthesis while minimizing water loss. Sorghum is a C4 plant adapted to moderate drought stress, and its extensive natural variation in photosynthetic traits can be used to develop stress-tolerant cultivars. The goal of this study was to understand the relationship between leaf functional traits and eco-physiological traits by combining phenomic, physiological, and genetic approaches. We monitored periodic rain-fed and rain-deprived conditions in the sorghum field and evaluated stress responses using stomatal gas conductance and relative water content, both of which were significantly reduced under rain-deprived conditions. Then, we phenotyped stomatal features (density, index, length, width, pore area, etc.), and leaf developmental traits, particularly specific leaf area and leaf thickness, as well as evaluating gas exchange and water use efficiency across natural variation present in sorghum accessions. The phenotypic data was integrated with genomic data and genome-wide association was performed to discover genes/genomic regions that control leaf functional and eco-physiological traits under natural conditions. Several candidate genes were identified with predicted functions related to stomata density and distribution, carotenoids, phytohormones, thioredoxin, components of PSI and PSII, and antioxidants. Further contrasting genotypes based on the REF and ALT alleles obtained from marker-trait analysis (SNPs for stomatal gas conductance and stomata density) will be utilized to model stomatal conductance in response to various CO2 concentrations. Overall, our findings contribute to our understanding of sorghum's natural variation and genetic control of leaf functional and eco-physiological traits in response to drought, with the ultimate goal of improving its adaptation and productivity under water stress conditions.

Funding acknowledgement: Department of Energy (DOE), MSU Plant Resilience Institute; AG2PI Seed Grant

T9 @mckenalwilson

Quantitative genetics of stress tolerance and agronomic traits in the climate resilient cereal teff

(submitted by McKena Wilson <liphamra@msu.edu>)
Full Author List: Wilson, McKena L[1]; VanBuren, Robert[1 2]
[1] Department of Horticulture, Michigan State University, East Lansing, MI 48824, USA
[2] Plant Resilience Institute, Michigan State University, East Lansing, MI 48824, USA

The underutilized cereal teff was domesticated in the highlands of Ethiopia where stability in arid conditions was selected for over yield. Teff has a high water use efficiency and can survive with minimal input of both water and fertilizer, making it an attractive alternative grain for production in low rainfall areas. We have developed the teff association panel to understand agronomic and resilience traits in this important cereal. This panel consists of 265 lines representing much of the global genetic diversity and includes wild varieties, landraces, and cultivars from the USDA-GRIN collection. The panel was surveyed in a multi-year field trial at Michigan State University and will be harnessed to characterize agronomic traits, develop molecular tools, and isolate high performing teff varieties. To evaluate the underlying molecular mechanisms of teff resilience, I designed and built twelve rainout shelters and simulated a climate relevant drought stress. Sixteen genotypes across all historical groups including the wild progenitor *Eragrostis pilosa* were phenotyped for drought tolerance and nutritional stability in the field. This intensive study combines phenotypic and transcriptomic datasets to evaluate teff drought tolerance and highlight the stability of teff production and seed nutrient content in a harsh climate. We aim to discover the underlying mechanisms of teff resilience and distinguish teff as a nutritious climate resilient cereal.

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

T10 @zhikai16

Microbiome-enabled genomic selection improves prediction accuracy for nitrogen-related traits in maize

(submitted by Zhikai Yang <zyang35@huskers.unl.edu>)
Full Author List: Yang, Zhikai[1 2]; Zhao, Tianjing[3]; Schachtman, Daniel[1 2]; Cheng, Hao[3]; Yang, Jinliang[1 2]
[1] Department of Agronomy and Horticulture, University of Nebraska-Lincoln, Lincoln, NE 68588, USA
[2] Center for Plant Science Innovation, University of Nebraska-Lincoln, Lincoln, NE 68583, USA
[3] Department of Animal Science, University of California Davis, Davis, CA 95616, USA

Root-associated microbiomes are increasingly known to play an important role in nutrient acquisition, stress tolerance, and disease resistance of plants. But it remains largely unclear to what extent these microbiomes contribute to trait variation for different genotypes and if their inclusion in the genomic selection (GS) protocol can enhance prediction accuracy.To address this, we developed a microbiome-enabled GS (MEGS) model that incorporated host SNPs and ASVs (amplicon sequence variants) from plant root-associated microbiomes in a maize diversity panel under high and low nitrogen (N) field conditions. Our results showed that the MEGS model significantly outperformed the conventional GS model for nearly all time-series traits related to plant growth and N responses, with an average relative improvement of 4%. This improvement was more significant for traits measured near the microbiome data collection date and was more pronounced under low N conditions, as some beneficial microbes can enhance N nutrient uptake, particularly in low N conditions. Our study also identified mediator microbes, such as *Massilia putida*, which are previously reported to promote plant growth under low N conditions. These large-effect ASVs or microbial agents could be applied as biofertilizers or soil additives to enhance crop performance for sustainable agriculture.

Funding acknowledgement: United States Department of Agriculture (USDA)

T11

Maize yield prediction accuracy increased by inclusion of genetics, environment, and management interactions with deep learning

(submitted by Daniel Kick <Daniel.Kick@usda.gov>)
Full Author List: Kick, DanielR[1 2]; Washburn, JacobD[1 2]
[1] USDA-ARS, Plant Genetics Research Unit, Columbia, MO
[2] Division of Plant Sciences, University of Missouri, Columbia, MO, USA

An organism's phenotype depends on genetic and environmental factors. The interplay between these ("GxE") can be complex and difficult to predict. Accurately accounting for these improves the efficacy of a crop improvement program, conservation initiative, or experimental design. We seek to better model GxE interactions with deep learning – which learns nuanced relationships from the data. We consider model performance relative to linear models and machine learning models and the benefits of including GxE. We find that linear models (specifically best linear unbiased predictors) incorporating weather data have the lowest prediction error (measured as root mean squared error, RMSE) on average (0.937 RMSE) while some model replicates perform much worse. Deep learning models are slightly less accurate on average (0.948 RMSE) but are much more consistent (approximately ¼th as variable, standard deviation of 0.013 vs 0.058). We find the inclusion of genetic and environmental data increases accuracy in deep learning models and removes spurious relationships in environmental data, leading to a more robust model. Lastly, we find that while that errors from poorly performing models are more tightly correlated than those of better performing models. This suggests that by combining diverse modeling techniques (e.g., best linear unbiased predictors and deep learning models), along with different types of data, allows for the shortcomings in one method to be compensated by the strengths of other models – resulting in more accurate predictions and better outcomes in agriculture, conservation, and basic research.

Funding acknowledgement: United States Department of Agriculture (USDA)

T12

Maize yield prediction: Results from the 2022 G2F prediction competition.

(submitted by Alencar Xavier <alencar.xavier@corteva.com>)
Full Author List: Xavier, Alencar[1 2]; Washburn, Jacob D.[3]; Barber, Wesley T.[1]; Zimmer, Cristiano[1]; Trucillo, Ignacio[1]; da Rocha, Fabiani[1]; de Leon, Natalia[4]; Holland, James[5]; Ertl, David[6]; Gage, Joseph[7]; Romay, Cinta[8]; Lima, Dayane[4]; Varela, Jose[4]; Qiuyue, Chen[7]
[1] Corteva Agrisciences; Johnston, IA, 50131, USA
[2] Department of Agronomy; Purdue University; West Lafayette, IN, 47907, USA
[3] USDA-ARS Plant Genetics Research Unit; University of Missouri; Columbia, MO, 65211, USA
[4] Department of Agronomy; University of Wisconsin; Madison, WI, 53706, USA
[5] USDA-ARS Plant Science Research Unit; North Carolina State University; Raleigh, NC, 27606, USA
[6] Iowa Corn Promotion Board; Johnston, IA, 50131, USA
[7] Department of Crop and Soil Sciences; North Carolina State University; Raleigh, NC, 27695, USA
[8] Institute for Genomic Diversity; Cornell University; Ithaca, NY, 14850, USA

Predicting maize yield in new environments and new genetics is notoriously difficult, but accurate prediction models have important ramifications for breeding efficiency and anticipating responses to climate change. Previous studies of yield prediction have relied on holding back results from certain environments as though they had not yet been observed, but the true test of a yield prediction model is its performance on data from completely new, never before seen environments. The maize Genomes to Fields (G2F) genotype by environment (GxE) project was conceived 10 years ago as a multi-state cooperative to test hypotheses about maize yield, adaptation, and environmental responses. To date the project has phenotyped around 180,000 unique plots, around 5,000 hybrids, and more than 280 environments. This year the GxE project held an international yield prediction competition using the complete G2F GxE project dataset to date, with the goal of predicting entirely unseen data collected in 2022. The competition attracted over 230 registrants from across the world with competitors from both the public and private sector. While many prediction strategies were submitted, the winning team used an Ensemble model containing two Genomic Best Linear Unbiased Prediction (GBLUP) strategies and utilized both genomic and environmental variables. This model resulted in an average normalized Root Mean Squared Error of 19.8% when compared to the range of observed values. This model represents the state of the art in yield prediction methods and establishes a new baseline for future work in this field.

Funding acknowledgement: National Corn Growers Association

T13

Spatial transcriptomic analysis of the maize embryo

(submitted by Hao Wu <hw388@cornell.edu>)
Full Author List: Wu, Hao[1]; Scanlon, Michael J.[1]
[1] School of Integrative Plant Science, Cornell University, Ithaca, NY 14853

Maize embryo development initiates the histological and morphogenetic patterns that are reiterated throughout shoot development. Diverse cell types, tissues, and organs are formed in a dynamic, spatial-temporal manner. Stage 1 is strategic for studies of embryo development, as the earliest timepoint when all types of lateral organs are formed (i.e. the scutellum, coleoptile and leaf). We are interested in determining the homology of the grass cotyledon and its relationship to foliar leaves. In this study we used laser microdissection RNAseq (LM-RNAseq), single-cell RNAseq (scRNA-seq), and spatial transcriptomics to profile the transcriptomic landscape of the Stage 1 embryo. The 10X Genomics Visium™ spatial transcriptomics protocol identified nine clusters that mapped to seven embryonic regions: SAM tip, leaf and coleoptile; lower SAM and hypocotyl; suspensor; apical internal scutellum; basal internal scutellum; adaxial scutellum epidermis; and the abaxial scutellum epidermis. In the spatial transcriptomic UMAP, cell clusters derived from the shoot-root axis are separated from those of the scutellum, whereas cells from the scutellum epidermis are distinct from cells derived from the internal scutellum. Gene ontology (GO) analyses suggest that the scutellum epidermis functions in intercellular transport. In contrast, cells from the internal scutellum display carbohydrate and/or fatty acid metabolic functions. To overcome the lower cell-resolution issues of the current Visium technology (i.e., 1-10 cells), we integrated these spatial-transcriptomic data with scRNA-seq and LM-RNAseq analyses of Stage 1 embryos to generate a transcriptomic consensus. Consistent with the Visium UMAP, the scRNA-seq UMAP also shows separation of the epidermal and internal scutellum cell clusters. The combined transcriptomic consensus reveals that cells from the foliar leaf and coleoptile cluster together, and are transcriptomically distinct from the scutellum and the suspensor. Moreover, higher *wox3* mutations have similar phenotypic effects on the leaf and coleoptile, which supports the proposed homology of these maize embryonic organs.

Funding acknowledgement: National Science Foundation (NSF)

T14

The peripheral (maternal) void network inside the maize kernel supports grain fill

(submitted by Hardy Rolletschek <rollet@ipk-gatersleben.de>)
Full Author List: Rolletschek, Hardy[1]; Xiao, Hui[2]; Verboven, Pieter[2]; Langer, Matthias[1]; Nicolai, Bart[2]; Borisjuk, Ljudmilla[1]
[1] Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Seeland-Gatersleben, Germany
[2] University of Leuven, BIOSYST-MeBioS, Leuven, Belgium

Hypoxia (oxygen deficiency) is known to affect gene expression, metabolic activities and developmental progression. Hypoxia was recently shown to arise naturally within the major part of developing endosperm, while the chalazal endosperm remains well oxygenated throughout the grain-filling phase (Langer et al., Plant Physiology, in press). We here investigated the mechanistic basis of hypoxia avoidance in the chalazal endosperm which includes the basal endosperm transfer layer, mediating assimilate transfer into endosperm. X-ray micro-computed tomography (µ-CT) was applied to immature B73 kernels, and uncovered a previously-unknown, but well-developed void space present in the basal pericarp region. The porous layer did not extend distally up the endosperm side of the kernel but did rise across the face of the developing embryo. A few small pores were evident in the embryo and these connected to the porous layer. No porous structures were found in central endosperm regions, indicating that voids were either completely absent or very small. The void space in pericarp was calculated to have a very high mean porosity of 34% (= volume of pore space/volume of whole tissue), which caused oxygen diffusivities five orders of magnitude higher than in central (hypoxic) endosperm. Further modelling revealed that 43% of all oxygen enters the endosperm via direct contact with sites of porous pericarp. To conclude, the peripheral (maternal) void network inside the maize kernel has high relevance for oxygen supply of basal endosperm (and embryo). It avoids hypoxia (*in sensu* energy limitation) in the basal endosperm transfer layer, and thereby supports grain fill.

T15

Liguleless narrow and *narrow odd dwarf* regulate maize development and stress response in overlapping pathways

(submitted by Jazmin Abraham-Juarez <jazmin.abraham@cinvestav.mx>)
Full Author List: Abraham-Juarez, Jazmin[1]; Busche, Michael[2]; Anderson, Alyssa[3]; Lunde, China[4]; Hake, Sarah[3]; Brunkard, Jacob[2]
[1] Langebio Cinvestav, Irapuato, Gto. México 36821
[2] University of Wisconsin, Madison, Wisconsin 53706
[3] Plant Gene Expression Center, USDA-ARS and UC Berkeley, Albany CA 94710
[4] University of California, Berkeley, California 94720

Liguleless narrow-R (*Lgn-R*) and *narrow odd dwarf* (*nod*) were identified in screens for leaf development mutants. Both phenotypes are dependent on inbred, in restrictive inbreds they are short with narrow leaves and altered proximal distal patterning. LGN encodes a kinase and the semi-dominant mutant lacks kinase activity. NOD encodes a plasma-membrane localized protein with MLKL and PLAC domains, that functions in calcium import. We carried out an immunoprecipitation- LC MS/MS analysis using a NOD specific antibody and identified LGN as one of the interacting proteins. The interaction was confirmed by BiFC in Nicotiana and by reciprocal CoIP in maize. In addition, LGN phosphorylates NOD *in vitro*, and the phosphorylated sites are those found *in vivo*. A phosphoproteome for *Lgn-R* and RNAseq of both mutants suggest they are autoimmune mutants, however, they affect distinct pathways. *Lgn-R* shows signs of PAMP-triggered immunity while *nod* shows signs of effector-triggered immunity. Indeed, the double mutant is more severe than either single mutant, supporting the idea they have different immunity outcomes. Transcriptomic analyses of the single and double mutants in two genetic backgrounds revealed widespread induction of pathogen defense genes. We suggest that LGN and NOD function together as a signaling hub to regulate leaf development and immunity. Future experiments using pathogen infections will reveal the molecular mechanism by which LGN and NOD work in immunity pathways in maize, and in other species by analyses of orthologs.

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

T16

Uncovering the genetic and metabolic bases of seed amino acid composition using a multi-omics integration approach

(submitted by Ruthie Angelovici <angelovicir@missouri.edu>)
Full Author List: Jenkins, Lauren[1]; Shrestha, Vivek[1]; Bagaza, Clement[1]; Yobi, Abou[1]; Chan On, Yen[1]; Ansaf, Huda[1]; Slaten, Marianne[1]; Fichman, Yosef[2]; Mittler, Ron[2]; Czymmek, Kirk[3]; Flint Garcia, Sherry[4]; Angelovici, Ruthie[1]
[1] Division of Biological Sciences, Interdisciplinary Plant Group, Christopher S. Bond Life Sciences Center, University of Missouri, Columbia, MO 65211, USA
[2] Department of Plant Science & Technology, University of Missouri, Columbia, MO 65211, USA.
[3] Donald Danforth Plant Science Center, Olivette, MO 63132.
[4] USDA-ARS, University of Missouri, Columbia, MO 65211, USA

Most crop seeds are deficient in the essential amino acids (EAAs), i.e., those that humans and livestock cannot synthesize themselves and must obtain from their diet. This is a problem because a diet lacking sufficient EAAs contributes to malnutrition and growth deficiencies. Unfortunately, efforts to improve the EAA profile of seed crops are continually stymied by the seeds themselves. A seed has an innate ability to "rebalance" its amino acid levels and composition back to a "normal" state in response to alterations (natural or engineered) to its protein composition. This proteome rebalancing mechanism, which is highly conserved across plant species, is responsible for the tight regulation of amino acids in crop seeds. While this mechanism is likely a useful coping strategy for seeds, it thwarts efforts to enhance the nutritional value of seed crops via breeding or engineering. Despite its ubiquity and its negative impact on such biofortification efforts, we know little about this mechanism at the molecular level. To shed light on the genetic and metabolic basis of this phenomenon, we have performed proteomics, metabolomics, and transcriptomics on seed storage mutants that were rebalanced in two model systems Arabidopsis and Maize, and compared them to the unperturbed genotypes. To further identify high-priority candidate genes, we have also integrated GWAS that were performed on both free and bound amino acids in both model systems. In addition to specific candidate genes, we have found strong evidence of the key role that ribosomal proteins play in determining/maintaining both amino acid levels and composition in seeds. We have also revealed that a tight redox homeostasis may be necessary for seed maturation and that seed storage proteins are key regulators of it. Altogether, our findings expose new insights of why and how seeds maintain proteomic composition plasticity, while preserving protein content and amino acid composition robustness. These insights can lead to new strategies in proteins and amino acid biofortification efforts.

Funding acknowledgement: National Science Foundation (NSF)

T17 @MateuszZelkows1

The meiotic crossover landscape in maize

(submitted by Mateusz Zelkowski <mz548@cornell.edu>)
Full Author List: Zelkowski, Mateusz[1]; Epstein, Ruth[1]; Wang, Minghui[1]; Zelkowska, Katarzyna[1]; Pawlowski, Wojciech[1]
[1] Cornell University, Ithaca, NY 14853, USA

Meiotic recombination is the main driver of genome evolution and a vehicle of plant breeding. In plants with large genomes, including maize, meiotic crossover (CO) events are predominantly located at distal chromosome sites. Molecular mechanisms responsible for this distribution pattern are not clear. To elucidate them, we developed a high-resolution method to identify the sites of COs and CO intermediates, which relies on detection of the MLH3 protein during meiotic prophase I. MLH3 is a key recombination protein engaged in the establishment and maintenance of CO intermediates in early prophase I and their resolution into COs at the end of prophase I. Identifying MLH3 locations in late prophase I allows for CO detection with high throughput and high accuracy, and is vastly superior to the conventional genetic marker-based CO detection approach. Tracking MLH3 locations throughout prophase I allowed us to follow CO site designation and identify that nucleosome occupancy patterns and DNA methylation are critical to this process. Using the *decreased in DNA methylation 1* (*ddm1*) and *chromomethylase 2* (*zmet2*) mutants we studied the impact of reduced nucleosome and DNA methylation on recombination patterns. We found that decreasing DNA methylation can increase the global CO number up to 70%, whereas changes in chromosome-wide nucleosome density redirect COs to pericentromeric sites that normally lack them. Furthermore, our data suggest that reduced DNA methylation decreases CO interference. We performed simulations to test the effect of the altered CO landscapes of the *ddm1* and *zmet2* mutants on breeding outcomes. Their results revealed significant boosts in both recurrent selection and introgression programs, showing the potential of applying the results of this work to plant breeding.

Funding acknowledgement: National Science Foundation (NSF)

T18

Circadian regulation of metabolomes and proteomes in maize heterosis

(submitted by Jeff Chen <zjchen@austin.utexas.edu>)
Full Author List: Ko, Dae Kwan[1]; Li, Zhi[1]; Harmon, Frank[2]; Chen, Z. Jeffrey[1]
[1] The University of Texas at Austin
[2] Plant Gene Expression Center and University of Califorina, Berkeley

Hybrid vigor or heterosis, is a widespread biological phenomenon that has translational impact on agriculture, food security, and human health. Heterosis refers to increased stature and fertility of the offspring that is superior to one or both parents. The phenomenon of heterosis has been extensively used in agriculture, and all corn grown in the U.S. is hybrid. However, the molecular basis for heterosis remains elusive. In Arabidopsis hybrids and allotetraploids, we uncovered a direct link of altered circadian rhythms to biomass heterosis. The more sugar and starch accumulate during the day, the more can be utilized and degraded at night to promote plant growth. This role of circadian regulation in heterosis is conserved in maize. Maize circadian regulators can complement mutant phenotypes in Arabidopsis. In maize, disruption of maize circadian clock genes reduces chlorophyll content and plant height. Chromatin immunoprecipitation sequencing (ChIP-seq) analysis reveals a temporal shift of maize clock protein targets to the early morning in the hybrids, leading to activation of morning-phased genes that promote photosynthesis and growth vigor. Moreover, metabolomes and proteomes that are involved in carbon assimilation are diurnally regulated and nonadditively accumulated in the hybrids. Compared with robust trait heterosis, metabolic heterosis is relatively mild. Most amino acids display negative mid-parent heterosis (MPH), while sugars, alcohols, and nucleoside metabolites show positive MPH. From the network perspective, metabolites in the photosynthetic pathway during the day show positive MPH in the hybrids, whereas metabolites in the photorespiratory pathway at night show negative MPH, which corresponds to nonadditive protein abundance and key enzyme activities in their respective pathways. Together, the data suggest that hybrids may more effectively remove toxic metabolites generated during photorespiration to maintain higher photosynthetic efficiency. The insights gained from these omics resources will help produce high-yielding hybrid maize and other crops.

Funding acknowledgement: National Science Foundation (NSF)

T19 @jrossibarra

Two teosintes made modern maize

(submitted by Jeffrey Ross-Ibarra <rossibarra@ucdavis.edu>)
Full Author List: Yang, Ning[1 2 3]; Wang, Yuebin[1 2];Liu, Xiangguo[4]; Yan, Jianbing[1 2]; Ross-Ibarra,Jeffrey[3 5 6]
[1] National Key Laboratory of Crop Genetic Improvement, Huazhong Agricultural University, Wuhan 430070, China
[2] Hubei Hongshan Laboratory, Wuhan 430070, China
[3] Department of Evolution and Ecology, University of California Davis, California 95616, USA
[4] Biotechnology Research Centre, Jilin Academy of Agricultural Sciences, Changchun 130033, China
[5] Center for Population Biology, University of California, Davis, CA 95616, USA
[6] Genome Center, University of California, Davis 95616, USA

Despite its global importance as a crop with broad economic, dietary, and cultural importance, the origins of maize and its closest wild relatives remained the topic of vigorous debate for nearly a century. Molecular analyses ultimately concluded that maize was domesticated once from a common ancestor with its closest extant relative, the lowland wild grass *Zea mays* ssp. *parviglumis*. But neither the current genetic model nor earlier models based on archaeological data account for the totality of available data, and recent work has highlighted the potential contribution of a second wild relative, the highland *Zea mays* ssp. *mexicana*. Here we present a detailed population genetic analysis of the contributions of both wild taxa to modern maize diversity using the largest sample of traditional maize varieties sequenced to date. We show that all modern maize can trace its origin to an ancient admixture event between domesticated ancient maize and *Zea mays* ssp. *mexicana* in the highlands of Mexico ca 5300 cal BP, some 4,000 years after domestication began. We show that variation in admixture is a key component of modern maize genetic and phenotypic diversity, both at the level of individual loci and as a factor driving a substantial component of additive genetic variation across a number of agronomic traits. Our results clarify the long-debated origin of modern maize, highlight the potential contributions of crop wild relatives to agronomic improvement, and raise new questions about the anthropogenic mechanisms underlying multiple waves of dispersal throughout the Americas.

Funding acknowledgement: National Science Foundation (NSF), United States Department of Agriculture (USDA), National Natural Science Foundation of China, Science and Technology, 111 Project Crop Genomics and Molecular Breeding major program of Hubei Province

T20 @ManishaMuna

Combined analysis of transposable elements and structural variation in maize genomes reveals genome contraction outpaces expansion

(submitted by Manisha Munasinghe <mmunasin@umn.edu>)
Full Author List: Munasinghe, Manisha A[1]; Read, Andrew C.[1 2]; Stitzer, Michelle C.[3]; Song, Baoxing[4]; Menard, Claire C.[2]; Ma, Kristy[5]; Brandvain, Yaniv[1 6]; Hirsch, Candice N.[2]; Springer, Nathan M.[1]
[1] Department of Plant and Microbial Biology, University of Minnesota, St. Paul, MN, 55108, USA
[2] Department of Agronomy and Plant Genetics, University of Minnesota, St. Paul, MN, 55108, USA
[3] Department of Molecular Biology and Genetics, Cornell University, Ithaca, NY 14853
[4] Peking University Institute of Advanced Agricultural Sciences, Weifang, 261200, China
[5] Department of Biology, Macalester College, St. Paul, MN, 55105, USA
[6] Department of Ecology, Evolution and Behavior, University of Minnesota, St. Paul, MN, 55108, USA

Structural differences between genomes are a major source of genetic variation that contribute to phenotypic differences. Transposable elements, mobile genetic sequences capable of propagating themselves within genomes, can generate structural variation. However, their repetitive nature makes it difficult to characterize fine-scale differences in their presence at specific positions, limiting our understanding of their impact on genome variation. Domesticated maize is a particularly good system for exploring the impact of transposable element (TE) proliferation as over 70% of the genome is annotated as TEs. High-quality TE annotations were recently generated for *de-novo* genome assemblies of 26 diverse inbred maize lines. We generated base-pair resolved pairwise alignments between the B73 maize reference genome and the remaining 25 inbred maize line assemblies. We then classified TEs as either shared or polymorphic in a given comparison. Our analysis uncovered substantial structural variation, representing both putative insertion and deletion events. Putative insertions in SNP depleted regions, which represent recently diverged identity by state blocks, suggest some TE families may still be active. However, our analysis reveals that, genome-wide, deletions of TEs account for more structural variation than insertions. These deletions are often large structural variants containing multiple TEs. Combined, our results highlight how TEs contribute to structural variation and demonstrate that deletion events are a major contributor to genomic differences.

Funding acknowledgement: National Science Foundation (NSF)

T21 @pabster212

Deciphering the evolutionary history of cell-type-specific accessible chromatin regions by comparative genomic approaches

(submitted by John Mendieta <jpm73279@uga.edu>)
Full Author List: Mendieta, John Pablo[1]; Schmitz, Robert[1]; Marrand, Alex[1]; Zhong, Silin[2]; Tu, Xiayou[2]
[1] Department of Genetics; University of Georgia, Athens, GA, 30605
[2] School of Life Sciences; University of Hong Kong, Hong Kong, 100871

Non-coding cis-regulatory elements (CREs) alter gene expression by recruitment of transcription factors and are implicated in plant development, stress response, and evolution. Recently, application of chromatin assays such as Assay for Transposase Accessible Chromatin (ATAC-seq) have enabled rapid genome-wide identification of accessible chromatin regions (ACRs), which harbor CREs. Additionally, advances in single-cell genomics simplify the deconvolution of cellular heterogeneity associated with plant tissues and enable the identification of cell-type-specific regulatory environments. Although these technologies put identification of cell-type resolved ACRs within reach, rapid evolutionary turnover of genomic sequence in plants makes understanding the function of any given ACR exceptionally challenging. Here, we combine single-cell resolved chromatin accessibility data with comparative genomics - conserved non-coding sequences (CNSs) - resulting in a powerful novel dataset and allowing us to investigate the importance of ACRs over evolutionary time. We generated and annotated single-cell indexed ATAC-seq datasets for Zea mays and its close relative Sorghum bicolor from leaf. Annotation of cell-types within each dataset enabled the identification of both cell-type-specific, and constitutive ACRs. A total of 79,064 ACRs were identified in Z. mays, with 69,112 being constitutive and 9,953 cell-type-specific ACRs in 10 cell types. In S. bicolor 86,153 ACRs were identified with 67,339 broadly accessible and 18,815 cell-type-specific ACRs in 9 cell-types. To understand their evolutionary history, ACRs were intersected with CNSs that we identified using PhastCons and the multi genome aligner progressive cactus. We found that in Z. mays, 1,881 (11%) of the cell-type-specific ACRs overlapped CNSs, as compared to 3,139 (16%) in S. bicolor. This novel analysis provides a glimpse into the importance of ACRs in a cell-type-resolved context, providing preliminary evidence for the conservation of ACR function in cell-type-specific context over 13 million years of evolution. Allowing further inquiry about the rate of gain and loss of cell-type-specific ACRs, and the importance of cell type in conservation of these regions.

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

T22 @mcstitzer

Elevated transposable element content is subtly associated with reduced fitness in maize

(submitted by Michelle Stitzer <mcs368@cornell.edu>)
Full Author List: Stitzer, Michelle C[1 2]; Khaipho-Burch, Merritt B[1 3]; Song, Baoxing[4]; Ramstein, Guillaume P[5]; Hudson, Asher I[6]; Feschotte, Cedric[2]; Buckler, Edward S[1 7]
[1] Institute for Genomic Diversity; Cornell University; Ithaca, NY, USA 14853
[2] Department of Molecular Biology and Genetics; Cornell University; Ithaca, NY, USA 14853
[3] Section of Plant Breeding and Genetics; Cornell University; Ithaca, NY, USA 14853
[4] National Key Laboratory of Wheat Improvement; Peking University Institute of Advanced Agricultural Sciences; Weifang, China 261325
[5] Center for Quantitative Genetics and Genomics; Aarhus University; Aarhus, Denmark, 8000
[6] Department of Entomology and Plant Pathology; NC State University; Raleigh, NC, USA 27695
[7] USDA-ARS; Ithaca, NY, USA 14853

Transposable elements (TEs) have long been proposed to generate deleterious effects to the survival and reproduction of their host genome when inserting into genes and regulatory sequences. Classical population genetic theory suggests an even greater cost to the host genome, with copy-number dependent selection against TEs. Such models have been difficult to interpret when applied to large genomes like maize, where there are hundreds of thousands of TE insertions that collectively make up 85% of the genome. Here, we use the Nested Association Mapping (NAM) panel, consisting of over 5,000 maize recombinant inbred lines (RILs). We project TE content from parental genomes to RILs, and measure segregating TE content. This varies by up to 100 megabases, and populations often show transgressive segregation in TE content. We use replicated phenotypes across numerous years and environments, to empirically measure the fitness costs of TEs. For an annual plant like maize, grain yield is not only a key agronomic phenotype, but also a direct measure of reproductive output. We find weak negative effects of TE accumulation on grain yield, nearing the limit of the efficacy of natural selection in maize. This results in a loss of one kernel (~0.1% of average per-plant yield) for every additional 14 megabases of TE content. We further test various genomic covariates that have been hypothesized as ways TEs affect their host genome, but find no impact of distance of TEs from genes, TE length, or TE methylation status on fitness. Instead, our findings are consistent with a model of selection on the additional bioenergetic cost of replicating this TE-derived DNA. Together, we provide rare empirical measurements of the fitness costs of TEs, and suggest that the TEs we see today in the genome have been filtered by selection against their deleterious consequences on maize fitness.

Funding acknowledgement: National Science Foundation (NSF)

T23 @aimeejschulz

reelGene: Fishing for good gene models with evolution and machine learning

(submitted by Aimee Schulz <ajs692@cornell.edu>)
Full Author List: Schulz, Aimee J[1]; Hsu, Sheng-Kai[2]; Wrightsman, Travis[1]; Zhai, Jingjing[2]; Miller, Zachary R[2]; AuBuchon-Elder, Taylor[4]; Kellogg, Elizabeth A[4]; Seetharam, Arun[5]; Hufford, Matthew B[5]; La, Thuy[2]; Romay, M Cinta[2]; Buckler, Edward S[1 2 3]; Monier, Brandon[2]
[1] Section of Plant Breeding and Genetics, Cornell University, Ithaca, NY USA 14853
[2] Institute for Genomic Diversity, Cornell University, Ithaca, NY USA 14853
[3] USDA-ARS; Ithaca, NY, USA 14853
[4] Donald Danforth Plant Science Center, St. Louis, MO USA 63132
[5] Department of Ecology, Evolution, and Organismal Biology, Iowa State University, Ames, IA USA, 50011

Assembled genomes and their associated annotations have transformed our study of gene function. However, each new assembly of a species generates new gene models. The maize reference genome has been annotated by both structural prediction methods and RNAseq evidence. With these annotation methods, we can see inaccuracies due to pseudogene misclassification, transposon activity, and intron retention from sequencing of unspliced transcripts. To evaluate the 1.5 million unique maize gene model predictions generated from the 26 NAM founder lines, we hypothesized that accurate gene models will be evolutionarily conserved. We assembled the gene space of over 400 Andropogoneae species (related to maize, sorghum, sugarcane, and *Miscanthus*) using short reads and collectively captured over one billion years of evolution.We developed a series of machine learning models that focused on the key transcription, translation, and splicing elements of a gene model. Our models compared sequence characteristics and conservation across the Andropogoneae species to predict whether each gene element was correctly annotated and showed evolutionary support. These predictions of gene elements were combined into another machine learning model, along with UTR, exon, and intron features, to determine whether an entire gene model was likely to produce a functional protein.This final model, reelGene, predicts pseudogenes, core and non-core genes, and genes showing proteome evidence across all maize gene models with over 90% accuracy. We find that 92% of the maize classical genes are predicted to be accurately annotated, while the same only applies to 5% of the B73 dispensable genes. These results highlight that gene model accuracy can be evaluated by leveraging a large cohort of related species. ReelGene will both provide a tool for studying gene models across taxa, including species that are only found in herbariums or have limited resources, and help improve gene model annotation for the maize community.

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

T24 @marcopxt

An R-package for cross prediction and optimization using genomic selection

(submitted by Marco Peixoto <deamorimpeixotom@ufl.edu>)
Full Author List: Peixoto, Marco Antônio[1 2]; Amadeu, Rodrigo[3]; Bhering, Leonardo Lopes[1]; Munoz, Patrício R.[4]; Ferrão, Felipe[4]; Resende, Márcio Fernado[2]
[1] Laboratório de Biometria, Universidade Federal de Viçosa, Viçosa, Minas Gerais State, Brazil
[2] Sweet Corn Breeding and Genomics Lab, University of Florida, Gainesville, Florida State, United States
[3] Bayer Crop Science, Chesterfield, Missouri State, United States
[4] Blueberry Breeding and Genomics Lab, University of Florida, Gainesville, Florida State, United States

Plant breeding programs rely on balancing long-term genetic diversity and genetic gains, which are conflicting goals. A method to deal with this corollary is through cross selection, where we can jointly optimize the selection of crosses and the maintenance of genetic diversity. The availability of genomic selection models enables the optimization take in account DNA marker effects and predict not only progeny means but also progeny variance. Given the problem detailed, here we described a flexible R package to cross prediction and optimization, leveraging genomic selection information and accommodates non-additive effects and multi-trait information. The package represents a tool for breeding programs to balance genetic gains and genetic loss (diversity). A valid mate plan is built based on two core aspects: (i) prediction of usefulness for potential cross and (ii) optimization of the set of crosses. Usefulness accounted for the mean and variance of each cross recovered from marker data, markers effects, and linkage disequilibrium matrix. The mean and variances can be predicted /estimated for traits with additive control or additive and dominance control. In addition, multi-trait scenarios are allowed by building a cross-selection index based on weights for each trait (for mean and variance of a cross). The optimization algorithm maximizes the usefulness and minimizes next generation inbreeding, which, at the end, circumvent the reduction of genetic diversity over breeding cycles by crossing less related individuals. The user may provide the ideal number of crosses, the maximum/minimum number of crosses per parent, and a maximum value of relationship between parents that can be used to create a cross. Then, it builds a mating plan from the target parental population. An example of implementation in a maize breeding program with two heterotic groups with a trait controlled by additive effect is given and highlights greater genetic gain over time.

Funding acknowledgement: United States Department of Agriculture (USDA)

T25

Single-cell transcript mapping in crop species using Molecular Cartography™

(submitted by Samik Bhattacharya
<samik.bhattacharya@resolvebiosciences.com>)
Full Author List: Bhattacharya, Samik[1]
[1] Resolve Biosciences GmbH, Alfred-Nobel-Str. 10, Monheim am Rhein, 40789, Germany

Genomic modulation of major crops, such as maize, is critical to solving some of the imminent challenges posed by global climate aberration threatening food security. However, a thorough understanding of contextual genetic pathways is inevitable to engineer high-yielding, resistant, and sustainable crops that can withstand biotic and abiotic stress. To decipher the underlying mechanism of molecular and physiological pathways that control agronomic traits, researchers have used NGS, adapted single-cell RNA-sequencing, and explored smFISH. While these techniques have boosted our understanding of molecular processes, they either lack the vital spatial information during the process of tissue dissociation into single cells or are limited by the number of genes analyzed at once, creating a 'spatial and throughput gap.' To address this, Molecular Cartography was developed to analyze up to 100 genes while working effectively with several crop species, including maize, and maintaining spatial context at a sub-cellular level. In addition, we present an overview of how Molecular Cartography, a high-resolution spatial transcriptomics platform, can revolutionize the contextual perception of crop genomics and help improve plant traits and genetics to tackle the planet's challenges.

Funding acknowledgement: Resolve Biosciences

T26

Streamline unsupervised machine learning to survey and graph indel-based haplotypes from pan-genomes

(submitted by Xianran Li <xianran.li@usda.gov>)
Full Author List: Zhang, Bosen[1]; Huang, Haiyan[1]; Tibbs-Cortes, Laura E.[2]; Vanous, Adam[2]; Zhang, Zhiwu[1]; Sanguinet, Karen[1]; Garland-Campbell, Kimberly A.[1 2]; Yu, Jianming[3]; Li, Xianran[1 2]
[1] Washington State University
[2] USDA-ARS
[3] Iowa State University

Pan-genomes with high quality *de novo* assemblies are shifting the paradigm of biology research. Large insertion and deletion (indel) polymorphisms, contributing to phenotypic variations thought altering gene structure or expression, are class of structural variants to be catalogued from pan-genomes. However, for specific genes, surveying and graphing large indels across assemblies are challenge and painstaking tasks. To overcome the challenge, we devised two unsupervised machine learning algorithms, CHOICE (Clustering HSPs for Ortholog Identification via Coordinates and Equivalence) and CLIPS (Clustering via Large-Indel Permuted Slopes). CHOICE automatically retrieves the segments harboring the ortholog from each assembly for the desired All-vs-All comparison while CLIPS groups accessions sharing same indels into haplotypes for concisely graphing the indel patterns. We then constructed an interactive webapp BRIDGEcereal (https://bridgecereal.scinet.usda.gov/) to expedite this process. Over hundred assemblies, including 26 maize NAM founder genomes and other cereal crops, were compiled into the database. With a gene model ID, or a transcript sequence, it only takes ~10 second to survey whether large indels are segregating among pan-genomes. Two adjustable parameters, up- and down-stream search boundaries, enable to survey the unknown sizes and locations for indels outside of gene body. We demonstrated that mining pan-genome through BRIDGEcereal could accelerate gene discovery and characterization with multiple maize and wheat genes. The versatile design enables to seamlessly incorporate newly released assemblies.

Funding acknowledgement: United States Department of Agriculture (USDA)

T27

Editing the 19kDa alpha-zein gene family generates non-opaque2 based quality protein maize

(submitted by Preston Hurst <jhurst5uwyo@gmail.com>)
Full Author List: Hurst, J. Preston[1]; Sato, Shirley[1]; Ferris, Tyler[1]; Yobi, Abou[2]; Clemente, Thomas E.[1]; Angelovici, Ruthie[2]; Holding, David R.[1]
[1] University of Nebraska - Lincoln; Lincoln, NE, USA 68503
[2] University of Missouri; Columbia, MO, USA 65211

Maize grain is deficient in lysine, an essential amino acid. Thus, livestock raised on maize based diets require supplementation. This poor protein quality is caused by the primary seed storage protein, the zeins, lacking lysine residues. Mutants of the opaque2 transcription factor (o2) have a reduction in zein proteins, and a corresponding increase in lysine rich non-zein proteins through a phenomoenon termed proteome rebalancing. Unfortunately, the alteration of kernel protein bodies in o2 produces a soft, chalky kernel unsuitable for broad production. Quality protein maize (QPM), developed at CIMMYT, utilized modifying loci which restored kernel hardness despite containing a mutant o2 allele. However, o2 regulates a wide network of genes, and pleiotropic effects have proved deleterious, with issues related to disease resistance as well as a collateral reduction in non-lysine amino acids. To address this problem, CRISPR/cas9 was used to directly edit a subfamily of the genes encoding zein proteins, the 19kDa alpha-zein subfamily. This rationale intends to address two issues with o2 based lysine enhancement: First, direct targeting allows for a reduction in zeins in the presence of a functional o2 transcription factor, and second, targeting only a specific family allows for proteome rebalancing to occur with less effect on kernel texture. Six gRNA target sites were used to edit all members of the 19kDa-alpha zein gene family. The resulting lines were outcrossed, and segregants of different edited haplotypes were assessed for amino acid content and kernel phenotype. The edited lines were found to have varying degrees of kernel hardness and amino acid content, depending on which loci contained edited alleles. Ultimately, the results demonstrated that editing the 19kDa-alpha zein gene family can produce maize with enhanced lysine and no collateral damage to non-lysine amino acids.

Funding acknowledgement: United States Department of Agriculture (USDA)

T28

Metabolic labeling of nascent RNA: a look into active transcription and stable vs. unstable transcripts in maize root tips

(submitted by Emily Wear <emily_wear@ncsu.edu>)
Full Author List: Wear, Emily E.[1]; Mickelson-Young, Leigh A.[1]; Urrutia, Joshua[2]; Song, Jawon[2]; Thompson, William F.[1]; Hanley-Bowdoin, Linda[1]
[1] North Carolina State University; Department of Plant and Microbial Biology; Raleigh, NC USA 27695
[2] University of Texas at Austin; Texas Advanced Computing Center; Austin, TX USA 78758

Transcriptional analysis by RNA-seq has become a foundational part of plant studies to understand gene regulation during development and responses to external conditions. However, very few studies in plants have explored the relationship between actual transcription and steady-state RNA abundance. A handful of studies have used Global nuclear Run-On sequencing (GRO-seq) or Precision nuclear Run-On sequencing (PRO-seq) to characterize nascent transcription in maize shoots, but these experiments involved isolating nuclei from cells before allowing bound RNA polymerases to "run-on" *in vitro*. In contrast, in this study, we used the cell-permeable, uracil analog 5-ethynyl uridine (EU) to metabolic label nascent RNA *in vivo* in intact maize B73 root tips. Following EU-labeling, nuclei were isolated and total nuclear RNA was extracted and depleted of ribosomal RNAs. EU-RNAs were biotinylated, affinity purified using streptavidin, and Illumina sequenced. We are currently comparing EU-labeled, nascent RNA reads to cellular and nuclear steady state RNA reads from root tips to examine active transcription dynamics, including retained intronic transcripts and other RNA processing intermediates, and stable versus unstable transcripts. A majority of genes expressed in root tips show 2-fold or greater changes in RNA levels between the nascent and cellular steady-state samples, highlighting the contrasting roles of degradation and accumulation as RNAs mature and are transported to the cytoplasm. We are also characterizing nascent transcripts found outside of annotated genes, many of which are in genomic locations where there are no reads in cellular steady state samples. Further investigation of nascent transcription in B73 and future comparisons to NC350 and Sorghum will broaden our understanding of transcriptional regulation and how it relates to DNA replication, chromatin structure, and other important processes in the plant cell.

Funding acknowledgement: National Science Foundation (NSF)

T29 @julien_roziere

Detection of preferentially located DNA motifs reveals distinct cis-regulatory sequences in gene-proximal regions of Arabidopsis thaliana and maize

(submitted by Julien Rozière <julien.roziere@inrae.fr>)
Full Author List: Rozière, Julien[1 2 3]; Samson, Franck[4]; Guichard, Cécile[1 2]; Correa, Margot[4]; Brunaud, Véronique[1 2]; Martin, Marie-Laure[1 2 5]; Coursol, Sylvie[3]
[1] Université Paris-Saclay, CNRS, INRAE, Univ Evry, Institute of Plant Sciences Paris-Saclay (IPS2), 91190, Gif sur Yvette, France
[2] Université de Paris Cité, Institute of Plant Sciences Paris-Saclay (IPS2), 91190, Gif sur Yvette, France
[3] Université Paris-Saclay, INRAE, AgroParisTech, Institut Jean-Pierre Bourgin (IJPB), 78000, Versailles, France
[4] Université d'Evry-Val-d'Essonne, Laboratoire de Mathématiques et de Modélisation d'Evry (LaMME), UMR CNRS 8071, ENSIIE, USC INRAE, 91000 Evry, France
[5] Université Paris-Saclay, INRAE, AgroParisTech, UMR MIA-Paris-Saclay, 91120, Palaiseau, France

With the development of high-throughput sequencing, the complete sequence of many genomes is now available. Nevertheless, one major challenge in genomics is decoding these raw sequences into useful information. In this context, one of the potential levers is the search for *cis*-regulatory elements, i.e., short DNA sequences controlling gene expression (Schmitz et al., 2022, doi:10.1093/plcell/koab281). These sequences are located in high density in the 5'- and 3'-proximal regions around the transcription start and termination sites, respectively. Although numerous experimental and computational studies have increased our knowledge of the proximal *cis*-regulatory sequences, their characterization remains incomplete. We implemented a genome-wide *de novo* method based on the assumption of topological constraints on the position of *cis*-regulatory sequences with respect to the transcription start or termination site. Using this approach, we provided a map of preferentially located motifs (PLMs) of *Arabidopsis thaliana* and maize that reveals the structure and function of proximal *cis*-regulatory sequences (Rozière et al., 2022, doi:10.3389/fpls.2022.976371). We report three types of PLMs in both proximal regions and emphasize conserved PLMs in both species, particularly in the 3'-proximal region. Comparison with resources from transcription factor and microRNA binding sites showed that 79% of the identified PLMs were unassigned, although some were supported by experimental chromatin accessibility data. Enrichment analyses also showed that unassigned PLMs provided functional predictions that differed from those derived from transcription factor- and microRNA-binding sites, highlighting the richness and diversity of PLMs. To finally facilitate the study of these proximal regions by plant biologists, we recently developed Plant-PLMview. This web-accessible database enables robust and accurate identification of PLMs in 20 plant genomes (Rozière et al., 2022, doi:10.1101/2022.12.20.521192). Plant-PLMview enables plant biologists to visualize proximal cis-regulatory sequences easily and opens opportunities for high-throughput genetic or functional studies related to adaptation to a changing environment.

Funding acknowledgement: Plant2Pro® Carnot Institute (agreement #19 CARN 0024 01), Saclay Plant Sciences-SPS (ANR-17-EUR-0007)

T30

Direct and predicted motif analysis of GRAS family transcription factors in Sorghum and other important crop species

(submitted by Nicholas Gladman <nicholas.gladman@usda.gov>)
Full Author List: Gladman, Nicholas P[1 2]; Kumari, Sunita[2]; Fahey, Audrey[2]; Regulski, Michael[2]; Ware, Doreen[1 2]
[1] USDA ARS NEA Plant Soil & Nutrition Laboratory Research Unit, Ithaca, NY, 14853
[2] Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 11724

Identifying non-coding regions that control gene expression has become an essential aspect of understanding gene regulatory networks that can play a role in crop improvements such as crop manipulation, stress response, and plant evolution. Recently, the expansion of high-quality reference genomes and chromatin profiling techniques have opened up direct and comparative accounting of transcription factor (TF) binding locations that could modulate proximal or distal gene expression. While a full complement of open chromatin, epigenetic, and TF-binding experiments provide information for likely candidate regulatory regions, using TF-binding approaches such as ChIP-seq or DAP-seq can provide additional valuable insight and targets for reverse genetic approaches such as EMS-induced or natural SNP variant screens or CRISPR editing techniques (e.g. promoter bashing). Here, we present the first ever DAP-seq profiles of three GRAS family TFs (SHR, SCL23, and SLC3) in the agriculturally important crop *Sorghum bicolor*. The binding location of the three GRAS TFs display unique and shared gene targets and categories of previously-characterized DNA-binding motifs as well as novel sequences that could potentially be GRAS family-specific recognition motifs and have been associated with gametogenesis, floral development, light signaling, hormone signaling, and root development. These results provide unique insight into the GRAS family of TFs and novel regulatory targets for further molecular characterization. This project was funded by the USDA-ARS award number 8062-21000-044-000D.

Gene / Gene Models described: *SHR, SCL23, SCL3*; SORBI_3001G327900, SORBI_3002G342800, SORBI_3005G029600

Funding acknowledgement: United States Department of Agriculture (USDA)

Posters

P1

A *WOX3*-patterning module organizes planar growth in grass leaves and ligules.

(submitted by Michael Scanlon <mjs298@cornell.edu>)
Full Author List: Satterlee, James W.[1]; Evans, Lukas[1]; Conlon, Brianne R.[1]; Conklin, Phillip[1]; Martinez-Gomez, Jesus[1]; Yen, Jeffery R.[1]; Wu, Hao[1]; Sylvester, Anne W.[1]; Specht, Chelsea D.[1]; Cheng, Jie[2 3]; Johnston, Robyn[1 4]; Coen, Enrico[2]; Scanlon, Michael J.[1]
[1] School of Integrative Plant Science, Cornell University, Ithaca, NY 14853
[2] John Innes Centre, Norwich Research Park, Norwich NR4 7UH, UK
[3] State Key Laboratory of Systematic and Evolutionary Botany, Chinese Academy of Sciences, Beijing 100093
[4] The Elshire Group Ltd., Palmerston North 4472, NZ

Grass leaves develop from a ring of primordial initial cells within the periphery of the shoot apical meristem (SAM), a pool of organogenic stem cells that generates all the organs of the plant shoot. At maturity, the grass leaf is a flattened, strap-like organ that comprises a proximal supportive sheath surrounding the stem, and a distal photosynthetic blade. The sheath and blade are partitioned by a hinge-like auricle and the ligule, a fringe of epidermally-derived tissue that grows from the adaxial (top) leaf surface. Together, the ligule and auricle comprise morphological novelties that are specific to grass leaves. Understanding how the planar outgrowth of grass leaves and their adjoining ligules is genetically controlled can yield insight into their evolutionary origins. Here we use single-cell RNA-seq (scRNA-seq) analyses to identify a "rim" cell-type present at the margins of maize leaf primordia, at the juxtaposition of adaxial-abaxial (top-bottom) leaf domains. Cells in the leaf rim have a distinctive identity and share transcriptional signatures with proliferating ligule cells, suggesting that a shared developmental genetic program patterns both leaves and ligules. Moreover, we show that rim function is regulated by genetically redundant WUSCHEL-LIKE HOMEOBOX3 (WOX3) transcription factors. Higher order mutations in maize *WOX3* genes greatly reduce leaf width and disrupt ligule outgrowth and patterning. Computational modeling of ligule outgrowth indicates that rim cell function at the juxtaposed domains of adaxial and abaxial cell identity, used to model mediolateral outgrowth from leaf margins, is also sufficient to grow a ligule. Together, these findings illustrate the generalizable use of a rim domain during planar growth of maize leaves and of ligules, and suggest a parsimonious model for the homology of the grass ligule as a distal extension of the sheath margin.

Gene / Gene Models described: *ns1, ns2, wox3a, wox3b, lg1*; Zm00001eb092480, Zm00001eb197430, Zm00001eb265710, Zm00001eb355310, Zm00001eb067740

Funding acknowledgement: National Science Foundation (NSF)

P2

A developmental boundary built by mutual microRNA mediated repression

(submitted by George Chuck <georgechuck@berkeley.edu>)
Full Author List: Dong, Zhaobin[1]; Shemyakina, Elena[1]; Ruggiero, Diana[2]; Leiboff, Sam[2]; Chuck, George[1]
[1] U.C. Berkeley, Albany CA, 94710
[2] Oregon State , Corvallis, OR, 97331

The formation of boundaries between adjacent fields with distinct identities is essential for all lateral development. For example, a boundary exists between the indeterminate cells of meristems and the determinate cells of the lateral organs initiated off their flanks. The establishment of this boundary allows unique developmental trajectories to occur between adjacent cells that occupy a common space with shared walls. When absent, lateral organ initiation and differentiation are compromised, and flowering and yield are greatly reduced. This can be observed in the floral boundary mutant *tasselsheath4* (*tsh4*) that initiates little or no tassel branches. How these boundaries are established is unknown, but it is clear that cell-to-cell communication between fields is essential to maintain them, and that developmental trajectories must be canalized for them to be made permanent. Our previous work uncovering the pathways downstream of the TSH4 transcription factor identified microRNA genes as direct targets that are negatively regulated. Interestingly, several of these microRNAs are known to negatively regulate *tsh4*, setting up a mutual auto-regulatory repressive loop. To understand how this is possible, we analyzed the timing and location of this mutual repression by simultaneously localizing the microRNAs and TSH4 protein. We show how the effects of this interaction are canalized by visualizing the *PIN1::YFP/DR5::RFP* auxin reporters in *tsh4* mutants. In sum, it appears that the microRNAs are expressed first and act as rheostats that modulate *tsh4* levels within overlapping domains. Once the boundary is formed, their relationship changes to one in which the microRNAs clear TSH4 proteins out of adjacent domains and vice versa. This boundary is later canalized by TSH4 that is known to target, and negatively regulate auxin response genes. The mutual repressive relationship between TSH4 and its microRNA targets explains how adjacent fields originating from a single primordium can be established during flowering.

Gene / Gene Models described: *tsh4*; Zm00001d020941
Funding acknowledgement: National Science Foundation (NSF), United States Department of Agriculture (USDA)

P3 @Xiaosa_Xu

A nuclear moonlighting function of RAMOSA3 in maize inflorescence branching

(submitted by Xiaosa Xu <xxu@cshl.edu>)
Full Author List: Xu, Xiaosa[1]; Claeys, Hannes[1 2]; Lang Vi, Son[1 3]; Lee, Jae Hyung[1]; George, Chuck[4]; Jackson, David[1]
[1] Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724, USA
[2] Present address: Inari, Industriepark 7A, 9052 Zwijnaarde, Belgium
[3] Present address: Agricultural Genetics Institute, Hanoi, Vietnam
[4] Plant Gene Expression Center, UC Berkeley, Albany, CA, 94710, USA

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. These candidates include a subtilisin-like serine protease gene with a potential function of regulating programmed cell death. Together, our data suggest that RA3 had a nuclear regulatory role in controlling inflorescence branching by interacting with the transcription factor, IDS1.

Gene / Gene Models described: *RAMOSA3, INDETERMINATE SPIKELET1*; GRMZM2G014729, GRMZM5G862109

Funding acknowledgement: National Science Foundation (NSF)

P4 @aribidopsis

A polarized nuclear position is required for correct division plane specification during maize stomatal development

(submitted by Arif Ashraf <arif.ashraf.opu@gmail.com>)
Full Author List: Ashraf, Arif[1]; Liu, Le[1 2]; Facette, Michelle[1]
[1] Department of Biology, University of Massachusetts Amherst, Amherst, MA, USA, 01003
[2] Plant Biology Graduate Program, University of Massachusetts Amherst, Amherst, MA, USA, 01003

Asymmetric cell division generates new cell types and is a feature of development in multicellular organisms. Prior to asymmetric cell division, cell polarity is established. *Zea mays* stomatal development serves as an excellent plant model system for asymmetric cell division, especially the asymmetric division of the subsidiary mother cell (SMC). In SMCs, the nucleus migrates to a polar location after the accumulation of polarly localized proteins, but before the appearance of the preprophase band. We examined a mutant of the outer nuclear membrane protein, which is part of the LINC (linker of nucleoskeleton and cytoskeleton) complex that localizes to the nuclear envelope in interphase cells. Previously, *mlks2* (*maize linc kash sine-like2*) was observed to have abnormal stomata. We confirmed and identified the precise defects that lead to abnormal asymmetric divisions. Proteins that are polarly localized in SMCs prior to division polarize normally in *mlks2*. However, polar localization of the nucleus is sometimes impaired, even in cells that have otherwise normal polarity. This leads to a misplaced preprophase band and atypical division planes. MLKS2 is localized to mitotic structures, however the structure of the preprophase band, spindle and phragmoplast appeared normal in *mlks2*. Timelapse imaging revealed that *mlks2* has defects in pre-mitotic nuclear migration towards the polarized site, and unstable position at the division site after formation of the preprophase band. We show that nuclear envelope proteins promote pre-mitotic nuclear migration and stable nuclear position, and that the position of the nucleus influences division plane establishment in asymmetrically dividing cells.

Gene / Gene Models described: *Mlks2*; GRMZM2G000608, Zm00001d052955

Funding acknowledgement: NSF, USDA, and UMass Amherst Start-up funding to Michelle Facette.

P5

Agrobacterium-mediated transformation of sweet corn inbred lines using morphogenic genes

(submitted by Vai Lor <vslor@wisc.edu>)
Full Author List: Lor, Vai S.[1]; Kaeppler, Shawn M.[1]
[1] University of Wisconsin - Wisconsin Crop Innovation Center, 8520 University Green, Middleton, WI 53562

Maize (*Zea mays* L) transformation technology has improved in recent years. Incorporation of the morphogenic genes *Baby boom* (*Bbm*) and *Wuschel2* (*Wus2*) greatly enhanced Agrobacterium-mediated transformation and expanded the range of inbreds that can be transformed. In this study, we tested if the morphogenic genes *Bbm/Wus2* can be used to generate transgenic sweet corn inbreds with edits in target genes. Sweet corn is largely based on three mutations affecting endosperm carbohydrate composition - *sugary1* (*su1*), *sugary enhancer1* (*se1*), and *shurnken2* (*sh2*) - supported by the proper background genetics. Three *su1*-type sweet corn inbreds (P39, IL14H, and W822SE) were chosen for this proof of transformation study. We also tested if transgenic sweet corn inbreds with CRISPR/Cas9-induced mutations can be generated. The *su1* gene in each sweet corn inbred was targeted using test gRNAs previously demonstrated in our lab to trigger Cas9-induced mutations in the *Su1* gene. In summary, we successfully generated transgenic sweet corn inbreds with *su1* Cas9-induced mutations in P39, IL14H, and W822SE using the morphogenic genes *Bbm/Wus2*.

Funding acknowledgement: United States Department of Agriculture (USDA)

P6

Analysis of FUN1 protein for identification of new factors involved in sex determination in maize

(submitted by Cristina Guerrero Méndez <cristina.guerrerom@cinvestav.mx>)
Full Author List: Guerrero-Méndez, Cristina[1]; Chuck, George[2]; Vajk, Angus[2]; Lunde, China[3]; Hake, Sarah[2]; Abraham-Juárez, M. Jazmín[1]
[1] Langebio Cinvestav, Irapuato, Gto. México 36821
[2] Plant Gene Expression Center, USDA-ARS and UC Berkeley, Albany CA 94710
[3] University of California, Berkeley, California 94720

Sexual determination in plants involves interaction between genes, environment and hormones. In maize there is a number of feminized mutants already studied, which are affected in different pathways including hormonal. In the present work we are analyzing the *fun1* (*feminized upright narrow 1*) EMS mutant, which shows a phenotype in both sex determination and leaf development. To investigate the function of the affected FUN1 protein in this mutant, we developed a specific antibody, which has been useful for immunolocalization, Western blot and immunoprecipitation. FUN1 is a highly disordered protein, with unknown function, it shows a low abundance in maize tissues with highest in inflorescences and endosperm. Protein localization shows an overlapping pattern with TS1, a lipoxygenase involved in JA signaling and sex determination, in inflorescences. In addition, we carried out a Y2H assay to identify candidates to interact with FUN1. Candidates related to hormonal pathways are being confirmed by BiFC. Hormonal levels in *fun1* compared to wt show altered levels of ABA, Jasmonic acid and ethylene. Our results suggest the interaction of FUN1 with floral development and hormonal pathways. These findings will allow reconstruction of the molecular pathways that follow sex determination in maize.

Gene / Gene Models described: *fun1*; Zm00001d039435

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

P7

Analysis of NARROW ODD DWARF in maize immune response

(submitted by Pablo Silva-Villatoro <pablo.silvav@cinvestav.mx>)
Full Author List: Silva-Villatoro, Pablo A.[1]; Hake, Sarah[2]; Abraham-Juárez, María J.[1]
[1] Langebio Cinvestav, Irapuato, Gto. México 36821
[2] Plant Gene Expression Center, USDA-ARS and UC Berkeley, Albany CA 94710

NARROW ODD DWARF (NOD) is a plasma membrane-localized maize protein, its ortholog in Arabidopsis is called Mid-Complementing Activity (MCA) and it has been reported to function in calcium import. The maize nod mutant shows an autoimmunity phenotype with a striking dependence on the genetic background. Transcriptome analysis of nod mutants revealed an overrepresentation of cell wall, hormone metabolism, and defense related gene categories. Plants have evolved an immune system that can be divided into two levels. Pattern recognition receptors (PRRs) recognize microbe-associated molecular patterns (MAMPs) promoting MAMP-triggered immunity (MTI). In response, pathogens have evolved effectors that repress or delay MTI. Plants have nucleotide-binding leucine-rich repeat receptor proteins (NLRs) which are activated directly or indirectly in the presence of pathogen effectors. Many NLR-mediated immune responses require NLR helpers (hNLRs). Some hNLRs have a 4-helix bundle (4HB) as part of the MLKL domain, this is required for pore formation in membranes and is known to produce cell death. NOD have the MLKL domain at the N-terminus, so, to test whether NOD can produce cell death in plant, we transiently expressed different versions of NOD protein in N. benthamiana, finding that NOD N-terminus domain is enough to produce cell death and that the change of a single amino acid in this domain abolish this function. To explore the nod involvement in pathogen response we are using Setosphaeria turcica, a maize fungal pathogen, to infect nod mutants in different genetic backgrounds and comparing to wild type plants. We found differential expression patterns of pathogenesis-related genes in B73 and Mo17, our findings suggest that NOD is one of the factors preventing PR genes induction in Mo17 which looks more resistant than B73 to fungi pathogens. Next experiments with fungi effectors will give information about NOD function at molecular level during pathogen infection in maize.

Gene / Gene Models described: *nod1*; Zm00001eb004320

Funding acknowledgement: National Science Foundation (NSF), UC-Mexus CONACYT

P8

Analysis of aberrant, preprophase band-independent TANGLED1 recruitment to the cell cortex in maize

(submitted by Aimee Uyehara <auyeh002@ucr.edu>)
Full Author List: Uyehara, Aimee N.[1]; Diep, Beatrice N.[1]; Allsman, Lindy A.[1]; Gayer, Sarah[1]; Kim, Janice J.[1]; Rasmussen, Carolyn G.[1]
[1] Department of Botany and Plant Sciences, Center for Plant Cell Biology, Institute for Integrative Genome Biology, University of California, Riverside, USA.

The orientation of cell division in plants is critical for proper growth and development. The preprophase band (PPB), a plant-specific cortical ring of microtubules and actin, predicts the future division site but disassembles before division is complete. Another microtubule structure called the phragmoplast promotes formation of the cell plate while expanding towards the location previously marked by the PPB. TANGLED1 (TAN1) is a microtubule binding protein and is among a small number of proteins that colocalize with the PPB and remain at the division site after PPB disassembly. Although the PPB has previously been shown to be required for TAN1 localization, here we show that TAN1 can be recruited to the cortex through a PPB-independent mechanism in maize. The maize double mutant discordia1 (dcd1) alternative discordia1 (add1) lacks a regulatory subunit of the PP2A phosphatase and does not make PPBs. Surprisingly TAN1-YFP in dcd1 add1 is recruited to the cell cortex late in division. To determine if TAN1 is recruited to the division site,TAN1 localization was observed in asymmetric divisions in the partial loss-of-function dcd1 single mutants, which sometimes makes defective PPBs. We observed TAN1 localization in aberrant de novo cell cortex locations, separate from TAN1 colocalization with mutant defective PPBs. Interestingly, TAN1 localization to the cortex in these aberrant locations seemed to follow the phragmoplast. To determine if aberrant TAN1 localization was due to defective PPBs or the phragmoplast, wild-type leaves were treated with CIPC, an herbicide that splits phragmoplasts but does not affect PPBs. TAN1 was observed at the cortex in aberrant locations where phragmoplasts ectopically contacted the cell cortex. These experiments suggest that TAN1 can be recruited to the cortex through a phragmoplast-based mechanism. Further experiments with chemicals that disrupt the cytoskeleton suggest that late TAN1 recruitment to the cell cortex does not depend on actin.

Funding acknowledgement: National Science Foundation (NSF)

P9

Characterization of mechanosensitive MSL gene family expression in Zea mays aerial and ground brace roots

(submitted by Olivia Hazelwood <oliviaha@udel.edu>)
Full Author List: Hazelwood, Olivia S.[1]; Hostetler, Ashley N.[1]; Ikiriko, Irene I.[1]; Sparks, Erin E.[1]
[1] Sparks Lab; University of Delaware; Newark, Delaware, USA 19711

To support a growing population, efforts should be made to improve our crops' resilience to external forces. Maize yield is decreased when the plant fails to stay upright due to a myriad of environmental factors in the field. The brace roots of maize are adventitious roots that aid in plant anchorage, but it is unknown if these roots can sense and adapt to mechanical stresses. Plants, similar to animals and bacteria, have the ability to sense mechanical stress from the environment, and contain a number of conserved Mechanosensitive (MS) ion channels. These ion channels receive mechanical input and in response can generate ion signals that allow the plant to adapt to its environment. In many important crops, such as maize, MS ion channels have been poorly characterized. Moreover, the MSL (standing for MscS-Like) gene family is responsible for stretch-gated MS ion channels that have a limited understanding in plants, especially in cereal crops such as maize. The focus of my study was to characterize the expression of the MSL gene family in the brace roots of different genotypes of maize. Preliminary analysis shows that the MSL gene family exhibits more variation and higher expression in the aerial brace roots compared to brace roots in contact with the soil. Future studies will investigate the functions of the MSL genes through mutant and electrophysiology experiments.

Gene / Gene Models described: *msl1, msl2, msl3, msl4, msl5, msl6, msl7, msl8*; Zm00001d029608, Zm00001d002580, Zm00001d002679, Zm00001d051415, Zm00001d017539, Zm00001d037120, Zm00001d044976, Zm00001d026135

Funding acknowledgement: National Science Foundation (NSF)

P10
Characterization of the floral development mutant *Polytypic ear1*

(submitted by Kimberly Rispress <rispressk04@students.ecu.edu>)
Full Author List: Rispress, Kimberly R[1]; Amoiroglou, Anastasia[1]; Thompson, Beth E[1]
[1] Department of Biology, East Carolina University, Greenville, NC, 27858, USA

Maize produces two inflorescences, the tassel and ear, which are critical for both plant reproduction and agriculture. The classical mutant, Polytypic ear1 (Pt1), affects multiple aspects of inflorescence development, including floral development. Interestingly, Pt1 appears to have roles in promoting and repressing meristem activity. Inflorescence primordia in Pt1/+ individuals often have fasciated inflorescence meristems and other meristems (i.e. spikelet pair, spikelet and floral meristems) are indeterminate, consistent with increased meristem activity. However, spikelet pair meristems often arrest in Pt1/+ inflorescences. In some genetic backgrounds, Pt1/Pt1 inflorescences almost completely lack lateral primordia, suggesting reduced meristem activity. To search for the causative gene in Pt1 mutants, we used a positional cloning approach to map Pt1 to a 5.3 Mbp region (6.8 cM) on chromosome 6, followed by RNA-seq to compare transcripts in Pt1/+ and normal ear primordia. From the 111 expressed genes in the Pt1-mapping interval, we identified 112 SNPs predicted to have a high or moderate effect on protein function. All 112 SNPs were also present in the HapMap and therefore unlikely to cause the Pt1 phenotype. We also identified nine genes in the Pt1-mapping interval that are differentially expressed in Pt1/+ and normal primordia. Pt1 is a semi-dominant mutant and thus likely a gain-of-function mutation. Therefore, Pt1 is more likely to be caused by one of the three upregulated genes. Our top candidate gene, ethylene receptor homolog3 (ETR3), encodes a putative ethylene receptor and shows allele-specific expression in Pt1. Ethylene receptors negatively regulate ethylene signaling, and in Arabidopsis, dominant mutations confer ethylene insensitivity. Overexpression of ethylene receptors may also confer a similar ethylene insensitive phenotype. To test the hypothesis that Pt1 is caused by etr3 overexpression, we are evaluating ethylene sensitivity of Pt1 mutants and examining etr3 transcript accumulation in Pt1 and normal plants using RNA in situ hybridization.

Gene / Gene Models described: *etr3*; Zm00001eb282800

P11 @PlantsOverPants
Comparing hormone dynamics in cereal crops via transient expression of hormone sensors

(submitted by Samuel Leiboff <leiboffs@oregonstate.edu>)
Full Author List: Dao, Thai[1]; Drapek, Colleen[2]; Jones, Alexander[2]; Leiboff, Samuel[1]
[1] Department of Botany and Plant Pathology; Oregon State University; Corvallis, Oregon USA 97331
[2] Sainsbury Laboratory; Cambridge University; Cambridge, CB2 1LR United Kingdom

Plant hormones are small molecules which elicit profound physiological responses. Although plant hormone biosynthesis and response genes have been critical for agricultural improvement, it has been difficult to experimentally compare hormone biology across species because of complex phenotypic outputs. We used transient expression of genetic hormone sensors and transcriptomics to quantify tissue-specific gibberellic acid (GA) and auxin responses across tissues and genotypes in cereal crops. We found that the FRET-based GPS2 sensor detects exogenous GA treatments in maize, barley, sorghum, and wheat, in both vegetative and floral tissues. Measuring GPS2 output across GA dosages revealed tissue- and genotype-specific differences in GA response. We observed marked differences in maize vs barley leaves vs floral tissues and an unexpected drop in GPS2 output in the maize *d1* GA biosynthesis mutant after GA treatment, likely reflecting differences in bioactive GA content and mechanisms of GA response. We then used RNAseq to measure transcriptional responses to GA treatment in leaves from maize wildtype, *d1*, and barley as well as floral tissues from maize and barley for a cross-tissue, cross-genotype, and cross-species GA-response comparison. After orthology prediction and analysis of within- and cross-species GO-term enrichment, we identified core sets of GA-responsive genes in each species as well as maize-barley orthogroups. Our analysis suggests that downregulation of *GA-INSENSITIVE DWARF1* (*GID1*) and upregulation of *α-Expansin1* (*EXPA1*) orthologs comprises a universal GA-response mechanism that is independent of GA biosynthesis, and identifies *F-Box*, *HEXOKINASE*, and *AMPK/SNF1 protein kinase* orthologs as unexpected cross-tissue, cross-genotype, and cross-species GA-responsive genes. We then compare the performance of transient expression of the DR5-DR5v2, and DII-mDII auxin sensor systems in barley and maize and find that although DR5 is not auxin-responsive in barley, DR5v2 responds to auxin treatment with a similar magnitude to maize. Both species display auxin-mediated DII degradation that requires the 26s proteasome.

Gene / Gene Models described: *EXPA1, d1, GID1*; Zm00001eb149460, Zm00001eb122500, Zm00001eb287800

Funding acknowledgement: National Science Foundation (NSF)

P12

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

(submitted by Penelope Lindsay <lindsay@cshl.edu>)
Full Author List: Lindsay, Penelope L[1]; Xu, Fang[1 2]; Boumpas, Panagiotis[1 3]; Liu, Lei[1 4]; Je, Byoung Il[1 5]; Skopelitis, Tara[1]; Jackson, David[1]
[1] Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724
[2] Shandong University, Jinan, China
[3] Heidelberg University, Heidelberg, Germany
[4] Huazhong Agricultural University, Wuhan, China
[5] Pusan National University, Miryang, South Korea

The maize ear is derived from a stem cell niche within the inflorescence meristem. Meristem size is controlled 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 synergistically with the *CLV* receptor mutants *td1* and *fea2*. Intriguingly, an ortholog of *TD1*, *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, indicating the two genes genetically interact. Furthermore, both FEA3 and ZmBAM1D perceive the same CLE peptide ligand. These observations indicate that FEA3 and ZmBAM1D may form a receptor-co-receptor pair. We are further exploring this interaction and discovering additional interactors of FEA3 and ZmBAM1D in maize inflorescence meristems using a proximity labeling approach called Turbo-ID, which can better resolve transient protein-protein interactions compared to immunoprecipitation-based approaches. With a deeper understanding of how these receptors regulate meristem activity, we can more precisely engineer this process to enhance yield-related traits.

Gene / Gene Models described: *fea3, bam1d*; Zm00001d040130, Zm00001d028317
Funding acknowledgement: National Science Foundation (NSF), United States Department of Agriculture (USDA)

P13

Cross-species analysis of gene regulatory circuitries underlying early inflorescence development in Panicoid grasses

(submitted by Edoardo Bertolini <ebertolini@danforthcenter.org>)
Full Author List: Bertolini, Edoardo[1]; Parvathaneni, Rajiv K.[1]; Braud, Max[1]; Yang, Jiani[1]; Eveland, Andrea L.[1]
[1] Donald Danforth Plant Science Center

Gene regulation is at the core of developmental plasticity contributing to plant adaptation and yield potential. Early events in inflorescence organogenesis directly influence seed production and yield, and therefore a comprehensive knowledgebase of gene-gene interactions and gene regulation during these developmental transitions will enable rational designs for fine-tuning inflorescence architecture and ultimately enhancing yield.In this work, we adopted a comparative study of early inflorescence development of three related species in the *Panicoideae* sub-family (*Zea mays*, *Sorghum bicolor* and *Setaria viridis*), each with morphological similarities and distinctions among them. We generated dynamic gene regulatory maps that describe early development of tassel/panicles at the molecular level in all three species. Plants were grown in environmentally controlled chambers and precisely-staged inflorescence primordia were hand-dissected at comparable stages associated with meristem fate and organ differentiation.RNA-seq analysis was performed across ~ 60 samples and integrated into gene regulatory and co-expression networks. In addition, genome-wide chromatin accessibility maps (ATAC-seq) were generated from key developmental stages capturing specific meristem types in each species. This constrained the networks by adding support for transcription factor (TF)-DNA binding predictions. These datasets were extended to include publicly available TF occupancy maps for important developmental regulators.Our integrated data analyses defined the transcriptional signatures, the molecular circuitries and their rewiring associated with early inflorescence development across closely related species. Together these data provide genome-scale insight into the coordinated regulation of gene expression during inflorescence development in the *Panicoideae*, which may be leveraged for targeted crop improvement.
Funding acknowledgement: National Science Foundation (NSF)

P14

Deciphering the molecular function of Sympathy for the ligule (Sol1) in development and maize immune response

(submitted by Rodrigo Muñoz Javier <rodrigo.munoz@cinvestav.mx>)
Full Author List: Muñoz-Javier, Rodrigo[1]; Anderson, Alyssa[2]; Hake, Sara[2]; Abraham-Juárez, Jazmin[1]
[1] Unidad de Genómica Avanzada, Laboratorio Nacional de Genómica para la Biodiversidad, CINVESTAV-Irapuato, Guanajuato, México 36821.
[2] Plant Gene Expression Center, USDA-ARS and UC Berkeley, Albany CA 94710.

Sympathy for the ligule (Sol1) was identified in previous studies as a maize ortholog of Arabidopsis ENHANCE DISEASE RESISTANCE 4 (EDR4). Sol1 encodes a MAPK-inhibiting protein EDR4-like and its activity is induced putatively during Pattern Triggered Immunity (PTI), in response to Pathogen Associated Molecular Patterns (PAMP). Existing evidence proposes that Sol1 possibly acts as a repressor of Liguleless narrow (Lgn) activity. Lgn triggers a MAP-kinase signaling cascade as a result of constitutive defense induction and the activation of biotic stress responses in mutants, which has a high developmental cost. However, the severe phenotype of Lgn is dependent on temperature and genetic background. This phenotype is restored by Sol1-Mo17 allele but not by the Sol1-B73 one, presumably by enhancing the Sol1 expression to repress the MAP-kinase signaling cascade. Based on transcriptomic, proteomic, and phenotypic analyses, we propose to dissect the molecular components subjacent to the genetic variation of Sol1 to elucidate regulatory changes of this genetic modifier, using the characterization of Sol1 mutants produced by CRISPR-Cas9. High genetic variation among maize inbreds is a valuable source to identify regulatory components of Sol1 activity and the molecular regulation required for the developmental control of phenotypes in Mo17 and B73. Likewise, to understand the function of Sol1 and mechanisms involved in the immune response under pathogen attack in maize.

Gene / Gene Models described: *Sol, Lgn*; GRMZM2G075262, GRMZM2G134382
Funding acknowledgement: National Science Foundation (NSF), UC MEXUS-CONACYT

P15

Dissecting cis-regulatory control of *ZmWUS1* expression through the binding of type-B response regulator proteins

(submitted by Zongliang Chen <zlchen@waksman.rutgers.edu>)
Full Author List: Chen, Zongliang[1]; Galli, Mary[1]; Gallavotti, Andrea[1 2]
[1] Waksman Institute of Microbiology, Rutgers University, Piscataway, NJ
[2] Department of Plant Biology, Rutgers University, New Brunswick, NJ

Mutations in *cis*-regulatory regions play a significant role in the domestication and improvement of crops by altering the expression of genes. However, assessing the impact of core *cis*-regulatory elements remains a challenging task. Previously, we have shown that the *Barren inflorescence3* (*Bif3*) mutant of maize contains a duplicated copy of the *ZmWUS1* gene, named *ZmWUS1-B*, expressed by a promoter that dramatically increases its expression and alters patterning and development of young inflorescences, in particular ears. Overexpression of *ZmWUS1B* is caused by a proximal enhancer containing multimerized binding sites for type-B RESPONSE REGULATORs (RRs), which are key transcription factors in cytokinin signaling. It is still unclear how this enhancer increases the expression of *ZmWUS1 in vivo*. To this end, we generated a CRISPR knockout construct specifically targeting the *ZmWUS1-B* enhancer region to create different deletion edits in the *Bif3* mutant locus. These edited events carry varying amounts of type-B RR DNA binding motifs (AGATAT), enabling us to examine how the number of AGATAT motifs impacts *in vivo* expression of *ZmWUS1-B* and ear development. We further utilized a dual luciferase assay in maize protoplasts to measure the effect of the number and spacing of AGATAT motifs on the expression of the *ZmWUS1* gene. Our analysis revealed that the presence of additional AGATAT motifs had an additive effect on the *ZmWUS1-B* promoter activity, while the distance between AGATAT motifs did not show a significant impact. These results not only expand our understanding of *cis*-regulatory logic on gene expression but the combination of AGATAT motifs found in the *Bif3* locus can be used to design an optimized cytokinin signaling marker line for monitoring cytokinin response in maize meristems.

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

P16 @whipplelab

Diverse mechanisms of tiller suppression in domesticated setaria and maize

(submitted by Clinton Whipple <whipple@byu.edu>)
Full Author List: Siddoway, Muriel[1]; Xiao, Yuguo[1]; Hu, Hao[2]; Doust, Andrew[2]; Whipple, Clinton[1]
[1] Brigham Young University, Department of Biology, 4102 LSB Provo, UT 84604
[2] Oklahoma State University, Department of Plant Biology, Ecology and Evolution, LSE 201 Stillwater, OK 74078

Lateral vegetative branches that grow at ground level in grasses are known as tillers, and regulation of tiller branching is important both agronomically and ecologically. Most cultivated cereals have reduced tillering compared to their wild ancestors and this is particularly apparent in panicoid cereals including maize, sorghum and setaria. Tillering in maize is almost entirely suppressed and is regulated by well the known domestication loci *teosinte branched1* (*tb1*). The loci responsible for reduced branching in domesticated sorghum and setaria are less well known, although *tb1* does not seem to be playing the central role it does in maize. Here we present the results of a morphological analysis of tiller growth and suppression in the wild setaria inbred A10 as well as two domesticated lines, B100 and yugu. While A10 buds grow continuously following germination, yugu initiates buds which become dormant after 16 days. Frequently B100 fails to initiate visible buds, suggesting this line has a distinct mechanism of bud suppression. Scanning Electron Microscopy of B100 bud development shows that buds are aborted in early stages. Surprisingly, we found a similar abortion of early stage tiller buds in the maize inbred A619. *tb1* mutants in A619 largely rescue the bud abortion indicating that *tb1* not only establishes tiller bud dormancy, but can also abort buds before they are fully formed. Thus, tiller branching can be regulated well before buds are formed in some lines suggesting a diversity of developmental strategies for tiller regulation in both maize and setaria. To further understand the genetic regulation of reduced tillering in domesticated setaria we explored transcriptomic changes associated with tiller suppression in A10, B100, yugu, and several A10 x B100 recombinant inbred lines by RNA-seq. The results of these transcript profiling experiments will be presented in light of the divergent mechanisms of tiller suppression in setaria.

Funding acknowledgement: National Science Foundation (NSF)

P17

Diversifying selection in evolution of the pleiotropic BIGE7/BRIZ E3-ligase

(submitted by Alec Chin-Quee <chinquee2@ufl.edu>)
Full Author List: Chin-Quee, Alec[1]; Wu, Shan[1]; Suzuki, Masaharu[1]; McCarty, Donald[1]
[1] Plant Molecular and Cellular Biology Program, Horticultural Sciences Department; University of Florida; Gainesville, FL 32611, USA.

Regulation of the cell cycle plays a central role in developmental transitions. Key transitions in maize include seed germination, aleurone differentiation, and gametogenesis. From the UniformMu population we identified *big embryo 7* (*bige7*) in a screen for mutations that increase embryo size at the expense of the endosperm. In addition, *bige7* has a distinctive suite of pleiotropic phenotypes. Aleurone differentiation is disturbed in the crown region of the endosperm. Mutant embryos, although fully formed at maturity, fail to germinate and suffer loss of cell-viability after imbibition. In addition to these kernel phenotypes, reciprocal crosses between wild type and *bige7* showed reduced male gametophyte transmission. Reduced pollen transmission implies function in male gametophyte development, whereas defects in Meiosis II division reveal an unusual partially dominant meiosis phenotype. Mu-seq analysis shows *Bige7* encodes a E3-ligase belonging to the highly conserved BRAP E3-ligase family implicated in cell cycle regulation in animals, and post-germination development in plants. Uniquely, flowering plants evolved a heterodimeric E3-ligase comprised of BIGE7/BRIZ1 and BRIZ2 subunits through gene duplication and sub-functionalization. Intriguingly, studies of the orthologous *briz1* mutant of Arabidopsis reveal only a subset of the pleiotropic *bige7* phenotypes suggesting BRIZ1 and BIGE7 roles have diversified in eudicot and monocot lineages. Hence, plant evolution provides a unique phylogenetic framework for dissection and comparative analysis of the poorly understood functions of BRAP E3-ligase in eukaryotic organisms. We have used phylogenetic analysis to determine the structural basis of sub-functionalization and functional diversification of BIGE7 and BRIZ proteins. Strikingly, we find evidence of diversifying selection at sites neighboring regions of protein and substrate interaction. To test the sites functional diversification, a series domain-swap hybrid proteins have been constructed for evaluation by transgenic complementation of *briz1* and *briz2* mutants in Arabidopsis. Diversifying selection suggests that BIGE7 co-evolves with targets in a changing molecular landscape.

Funding acknowledgement: National Science Foundation (NSF)

P18 @ireneikiriko

Does stalk flexural stiffness require cellular-scale mechanosensing?

(submitted by Irene Ikiriko <iikiriko@udel.edu>)
Full Author List: Ikiriko, Irene I.[1]; Hostetler, Ashley N.[1]; Sparks, Erin E.[1]
[1] Department of Plant and Soil Sciences, University of Delaware, Newark, DE 19713, USA

Plant mechanical failure, known as lodging, adversely impacts crop yields. In the United States, lodging accounts for 5-25% reduction in maize yields annually. One way to limit yield loss due to lodging is to determine how plants perceive and respond to dynamic forces (e.g. wind). Using maize inbred line CML258, we first defined how plant biomechanics (i.e. stalk flexural stiffness) changes during vegetative and reproductive development. Our results show that in CML258, flexural stiffness (EI) increases as plants reach reproductive maturity. We hypothesized that these changes in EI are due to cellular-scale mechanosensing and responses. Plants transduce mechanical signals through mechanosensitive proteins, of which, one well studied and characterized family is the MScS-Like (MSL). We used putative maize MSL mutants to determine if changes in EI are associated with cellular-scale mechanosensing. Results from MSL4 and MSL5 mutants show that the wildtype plants have a higher EI than their mutant counterparts, thus providing initial support for our hypothesis. Together, these data provide a basis for understanding lifespan-related changes in biomechanics as an outcome of cellular-scale mechanosensing and provide insight into breeding mechanically resilient crops.

Funding acknowledgement: National Science Foundation (NSF)

P19 @EgesaAO

Effect of heat stress on reproductive development in *Zea mays* L

(submitted by Andrew Egesa <egesaa@ufl.edu>)
Full Author List: Egesa, Andrew Ogolla[1]; Dresselhaus, Thomas[2]; Begcy, Kevin[1 3]
[1] University of Florida, Environmental Horticulture Department; Gainesville, Florida, USA, 32611-0670.
[2] Cell Biology and Plant Biochemistry, Biochemie-Zentrum Regensburg, University of Regensburg; Regensburg, Germany, 93053
[3] Plant Molecular and Cellular Biology Graduate Program, University of Florida; Gainesville, Florida, USA, 32611

Major shifts in the duration and intensity of ambient temperature affects plant development and reproduction. In maize, pollen development is especially sensitive to abiotic and biotic stresses. Therefore, it is imperative to analyze existing adaptive responses that can be leveraged in breeding strategies for stress tolerance in crops such maize to make them more resilient to climate change challenges accelerated by global warming. Using the *Leaf Collar Method*, we tracked pollen stages allowing us to study and dissect environmental responses on discrete pollen developmental stages. To understand how heat stress impacts the tricellular stage of pollen development in *Z. mays*. B73 maize plants were subjected to moderate heat stress (35ºC/25ºC, day/night) for 48 hours, while a similar set of control plants were maintained control temperatures (25ºC/21ºC). Heat stress hastened the development of maize anthers resulting in improper maturation and reduction in pollen dehiscence. Apart from the downregulation of the late pollen gene (*ZmMADS2*), known to aid anther dehiscence, we observed altered synthesis of flavonols and anthocyanins in anthers of the heat-exposed maize plants. Furthermore, we found altered gene expression patterns in pollen grains on genes coding for proteins such as Kinase 20, profilins, and plastocyanin which may be critical in pollen germination. Evidence from other crops suggests that anther indehiscence due to heat stress is caused by impaired moisture flow and sugar metabolism. A similar scenario may be actual in maize anthers. However, our findings indicate that secondary metabolism may also have a critical role in this process. Therefore, these findings point out the need to investigate the role of secondary metabolism in anther dehiscence as well as the necessity to evaluate other maize genotypes to understand how they respond to heat stress to select for and to breed maize plants tolerant to heat stress.

Funding acknowledgement: DAAD, BayKlimaFit

P20

Electric field treatment induces chromosome doubling in maize cell culture

(submitted by Rachel Egger <rachel.egger@syngenta.com>)
Full Author List: Egger, Rachel L.[1]; Crawford, Travis D.[2]; Burch, Erin[1]; Mahajan, Shivani[2]; Kinsey, Kayla[1]; Zhang, Cankui[2]; Garner, Allen[2]
[1] Seeds Research, Syngenta, Research Triangle Park, NC, 27709 USA
[2] Purdue University, West Lafayette, IN 47906 USA

Doubled haploid (DH) technology creates thousands of new inbred lines annually on a global scale and has become a key tool for maize hybrid breeding programs. DH production requires chemical doubling agents to induce genome doubling and restore male fertility, in the absence of which, maize haploids are typically 100% male sterile. But such chemical interventions are stressful to the plant and highly toxic; therefore there exists an opportunity for alternative doubling methods to increase doubling efficiencies in haploids. Working from the concept of tumor-treating fields (TT-fields), which are used clinically to lethally disrupt mitosis in cancer cells, we sought to test whether low-frequency electromagnetic fields could disrupt mitosis in plant cells and, if so, whether chromosome doubling would result from that disruption. Our results indicate that there exists a narrow band of frequencies at which mitotic disruption, and a subsequent doubling of genome content, can be induced in black Mexican sweetcorn (BMS) suspension cells grown in liquid culture – a completely new mode of action for doubling in plant species.

Funding acknowledgement: Syngenta Seeds Research

P21

Engineering root system architecture to improve teh water use efficiency of grasses

(submitted by Héctor H. Torres-Martínez <hhtormar@stanford.edu>)
Full Author List: Torres-Martínez, Héctor H.[1]; Brophy, Jennifer A. N.[2]; Tamborski, Janina[2]; Viana, William G.[1]; Clarke, Taylor[1]; Dinneny, José R.[1]
[1] Department of Biology, Stanford University, Stanford, CA 94305, USA
[2] Department of Bioengineering, Stanford University, Stanford, CA 94305, USA

Due to the vital role of cereal crops for human sustenance, understanding what developmental mechanisms allow grass species to acclimate to water-limited agricultural conditions is crucial. In grasses, shoot-borne roots termed crown roots act as an important interface between the plant and soil water, especially in adult plants. In this project, we propose using novel genetic engineering methods to quantitatively control the root system architecture of the model grass species *Setaria viridis* by manipulating the developmental pathways controlling crown root initiation. This approach will help to understand the critical role of crown roots in shaping root system architecture and its performance under drought conditions. Our lab has established a synthetic biology toolkit that can be used to assemble synthetic gene circuits to finely tune the expression of candidate genes in time and space. We will tune the expression of genes specifically expressed during crown root primordium development, resulting in plants with different numbers of crown roots. To analyze the architectures of different engineered plants, we will use GLObot. This robotic system will help to analyze the crown root development dynamics in both well-watered and drought conditions. GLObot allows automatic root system imaging in plants that constitutively expressed luciferase within rhizotrons where watering can be precisely controlled. Finally, we will evaluate parameters associated with water content in aerial parts to validate the performance of engineered plants under different water availability conditions. Overall, this investigation will shed light on how specifically and quantitively controlled CR development can help plants survive under drought conditions. This strategy may be extended to other economically useful grass species to deal with environmental stresses. It may help overcome issues associated with traditional crop improvement, including the lack of precision in enhancing a desirable trait.

Gene / Gene Models described: ; Setaria viridis v2.1

Funding acknowledgement: Department of Energy (DOE)

P22 @williangviana

Exploring a new pathway for crown root development in grasses

(submitted by Willian Goudinho Viana <viana@stanford.edu>)
Full Author List: Goudinho Viana, Willian[1]; Sebastian, Jose[2]; Scharwies, Johannes Daniel[1]; Clarke, Taylor L.[1]; Duncan, Keith E.[3]; Topp, Christopher N.[3]; Jiang, Hui[3]; Starker, Colby[4]; Dinneny, José R.[1]
[1] Stanford University, Stanford, CA, USA 94305
[2] Indian Institute of Science Education and Research, Berhampur, Odisha, India 760010
[3] Donald Danforth Plant Science Center, St. Louis, MO, USA 63132
[4] University of Minnesota, Saint Paul, MN, USA 55108

The mature root system in grasses is primarily composed of crown roots, which are essential for anchorage and acquiring water and nutrients. Despite their importance in crop productivity, the genetic mechanisms behind crown root development in response to environmental factors are not well understood. We have demonstrated in the panicoid grass model Setaria viridis that soil moisture around the crown stimulates the development of crown roots, while drought conditions inhibit their growth, enabling the plant to conserve water stored in the soil. We have isolated a mutant named crown root defective-1 (crd-1) that specifically impairs crown root development under well-watered conditions. Interestingly, rewatering after drought stress results in the rescue of the mutant phenotype. Notably, this response in crd-1 is temporary, and the mutant eventually stops making crown roots if it continues to grow in well-watered conditions. We used Bulk Segregant Analysis to fine-map the mutation and identified a single SNP disrupting the splice site of a gene encoding a WD-repeat protein (WDR6), which results in a premature stop codon in the coding sequence. Using CRISPR/Cas9 gene editing, we generated secondary alleles and found that they exhibit a crown root phenotype identical to that of crd-1. In addition, we complemented the crd-1 mutant with the wild-type sequence and found that it successfully rescued the mutant phenotype to wild-type levels. WD-repeat proteins can act as scaffold proteins that organize multi-protein complexes and can also regulate gene expression. To understand the function of the WDR6 protein, we performed a yeast-two hybrid screen to identify its binding partners. This approach led to the discovery of several binding partners, including members of the Growth-Regulating Factor family of transcription factors. We are now working on validating these interactions and planning to conduct functional studies to further explore the role of the WDR6 protein.

Funding acknowledgement: Department of Energy (DOE), Stanford University

P23

Function-value trait modeling of computer vision-derived epidermal cell patterning data to reveal the genetic basis of stomatagenesis in C4 grasses

(submitted by John Hodge <jgerardhodge@gmail.com>)
Full Author List: Hodge, John G[1]; Leakey, Andrew DB[1 2 3 4]
[1] Center for Advanced Bioenergy and Bioproducts Innovation, UIUC; 1206 Gregory Drive, Urbana, IL, 61801
[2] Departments of Plant Biology and Crop Science, UIUC
[3] Institute for Genomic Biology, UIUC; 1206 Gregory Drive, Urbana, IL, 61801
[4] Center for Digital Agriculture, UIUC

Water use efficiency (WUE), the ratio of photosynthetic CO_2 uptake relative to water vapor loss by transpiration, is a key target for crop improvement. Stomata play a key role in regulating WUE and are a model system for studying the mechanisms controlling cell fate and patterning. The density of stomata on the leaf surface is a complex product of the proportion of cells that acquire stomatal fate, the size of stomatal complexes, and the size of pavement cells that surround stomata. But, as is common for complex traits, a flexible framework to holistically evaluate the component traits of stomatal patterning, and the genotype-to-phenotype associations underpinning them, is still lacking. Recent application of machine-learning analysis to optical tomography data have provided unprecedented detail on the identities, numbers, sizes, and relative positions of epidermal cells, including stomata and underlying veins. Here we develop and apply a function-value trait (FVT) model of stomatal patterning to exploit new, rich phenotypic datasets. The model decomposes cell patterning into a reduced set of key parameters that are based on well-established understanding of developmental mechanisms underpinning stomatagenesis. Cell patterning is an ideal model system due to the zero-sum nature of cell packing and the constraints it places on relationships among component traits. The FVT model successfully simulated epidermal development to recapitulate variation in stomatal patterning within a maize B73 x MS71 RIL population, as well as in sugarcane and sorghum. We present this in terms of nearest neighbor analysis of Manhattan and Euclidean distances, as well as model-generated graphic representations of epidermal cell patterning. We are currently comparing QTL analysis of FVTs versus traditional stomatal patterning traits (e.g. stomatal index) and WUE traits. Overall, this provides a case study for FVT modeling as an approach to understanding interrelated complex traits.
Funding acknowledgement: Department of Energy (DOE)

P24

Functional interrogation of pollen-expressed genes using the *Ds-GFP* insertional mutant population

(submitted by John Fowler <fowlerjo@oregonstate.edu>)
Full Author List: Vejlupkova, Zuzana[1]; Bang, MIchelle[2]; Bell, Harrison[1]; Flieg, Harrison[1]; Garcia-Lamas, Luis[2]; Leary, Marilyn[1]; Ruggiero, Diana[1]; Leiboff, Sam[1]; Jiang, Duo[2]; Megraw, Molly[1]; Fowler, John[1]
[1] Department of Botany & Plant Pathology, Oregon State University, Corvallis, OR USA 97331
[2] Department of Statistics, Oregon State University, Corvallis, OR USA 97331

The male gametophyte of flowering plants, primarily visible as pollen, is required for sexual reproduction. It delivers sperm cells to the female gametophyte for double fertilization, which enables the subsequent development of the seed. Due to its haploid nature, mutations that affect pollen function result in a quantitative phenotypic effect on pollen fitness. This is detectable when outcrossed from a heterozygous parent as a transmission rate that differs from the Mendelian 1:1 ratio. In maize, a large set of fluorescently-marked insertional mutations, the *Ds-GFP* lines (acdsinsertions.org), provides a powerful tool for measuring the effect of single gene knockout mutations in the gametophyte by determining the ratio of mutant (Green Fluorescent Protein-marked) to wild-type progeny kernels in reciprocal outcrosses. We are using this resource to measure gene-specific contributions to pollen fitness for ~300 genes identified as expressed in mature pollen or sperm cells via transcriptomic or proteomic data. The EarVision phenotyping pipeline combines a digital imaging platform that enables visualization of the entire maize ear with a deep learning model that automates kernel categorization, increasing throughput for quantitative assessment of *Ds-GFP*-linked transmission defects. Through the 2021 field season, we have identified 20 insertion alleles associated with non-Mendelian segregation using a GLM model. Among these are several alleles that appear to be responsive to pollen load, transmitting more frequently when pollen competition is decreased. Also notable are three insertions associated with the so-called dog-in-the-manger (dim) phenotype, where the mutation in the pollen leads to increased frequency of ovules with no apparent seed development. Finally, we are validating insertion sites via PCR and Sanger sequencing, generating a gold-standard collection of precisely mapped kernel markers distributed throughout the genome, available for community use.

Funding acknowledgement: National Science Foundation (NSF)

P25

Genetic basis of tillering in sorghum

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

Tillers are axillary branches that form from compressed nodes near the ground level in grasses. Tillering is a highly plastic developmental process and influenced by internal signals and a wide range of external stimuli, such as water and nutrient availability, and light quality. Understanding the genetic signals that regulate tillering is of critical importance to optimizing yield potential in the world's major food, animal feed and biofuel grass crops. Sorghum [*Sorghum bicolor* (L.) Moench], an important cereal crop worldwide and emerging bioenergy feedstock, produces zero to many tillers in field conditions depending on its genotype and local growth environment. Here, we integrate approaches in genetics, molecular biology and multi-omics to explore the genetic basis for tillering in sorghum. We screened a chemically-mutagenized population of the grain sorghum BTx623 and identified several mutants with enhanced tillering phenotypes. Using bulked segregant analysis sequencing, we identified putative causal mutations responsible for the tillering mutant phenotypes. We are also characterizing the functions of sorghum orthologs of tillering genes identified in other cereals such as *grassy tillers1* (*gt1*) from maize. Our data show that loss of function of sorghum *GT1* (*SbGT1*) created by *CRISPR/Cas9* gene editing resulted in significantly increased tillering, specifically when grow in shaded conditions, indicating that *SbGT1* is a critical player in shade-induced tillering inhibition. In addition, we are applying multi-omics methods to build an integrative framework to predict genes and molecular networks necessary for tillering regulation in sorghum. Our prelimlary results suggest that SbGT1 directly regulates the expression of a suite of genes, including those known to be involved in abiotic stress response. Taken together, our project elucidates the genetic components and regulatory framework modulating sorghum tillering in response to internal and external cues, which can be leveraged to facilitate breeding of cereal crops with enhanced biomass or increased resilience to limited resource availability.

Gene / Gene Models described: *gt1*; GRMZM2G005624
Funding acknowledgement: Department of Energy (DOE)

P26 @AminaChaudhri

Genetic dissection of maize shoot and inflorescence architecture

(submitted by Amina Chaudhry <amina.chaudhry@rutgers.edu>)
Full Author List: Chaudhry, Amina[1]; Galli, Mary[1]; Gregory, Jason[1]; Chen, Zongliang[1]; Schimtz, Robert[2]; Gallavotti, Andrea[1]
[1] Waksman Institute of Microbiology, Rutgers University, Piscataway, NJ 08540, USA
[2] Department of Genetics, University of Georgia, Athens, GA 30602, USA

Changes in plant architecture are known to be associated with genetic variation either in the coding sequence or regulatory regions of several well characterized genes. Notable changes in plant and inflorescence architecture characterize mutants of maize domestication loci, such as *grassy tillers 1 (gt1), teosinte branched 1 (tb1)*, and *enhancer of tb1 (etb1.2).* Using a forward genetics screen to identify additional architecture-related genes, a novel mutant, *eary,* was isolated in an enhancer EMS-mutagenesis screen of the *ba1-mum1* mutant. Homozygous *eary* mutants in the A619 inbred line are short plants bearing 4-6 ears atop of extremely elongated shanks, while internodes and tassels are significantly shorter than in wild type plants. A candidate gene was identified based on a Glu>Lys missense mutation of a highly conserved amino acid within the coding region of a ferric-chelate reductase gene. However, in subsequent generations, we witnessed the disappearance of the *eary* phenotype. Therefore, we hypothesize that *eary* is a conditional mutant that regulates lateral branching in maize. Currently we are trying to identify the environmental factors involved in this cryptic phenotype. Concomitantly, we are dissecting upstream regulatory regions in two genes modulating maize shoot and inflorescence architecture, *TEOSINTE GLUME ARCHITECTURE1* (*TGA1*) and *TASSEL SHEATH1* (*TSH1*). Using a combination of DAP-seq and ATAC-seq datasets, we identified numerous transcription factor-binding events in regions of open chromatin that may act as cis-regulatory modules within the promoter of each gene. These regions were targeted by multiplexed CRISPR-Cas9 editing to parse out their potential regulatory function and fine-tune specific architectural traits. NSF IOS# 1546873, 1916804

Funding acknowledgement: National Science Foundation (NSF), Fulbright Pakistan

P27

Genetic networks underlying unisexual flower development in maize

(submitted by Comfort Bonney Arku <cbonneyarku@umass.edu>)
Full Author List: Bonney Arku, Comfort B[1]; Bartlett, Madelaine[1]; Deneve, Amber[1]
[1] University of Massachusetts, Amherst, Massachusetts, USA, 01003

Flowers vary in their form and structure. One of the important developmental processes that drives this variation is growth suppression. In maize, unisexual flowers develop through differences in the suppression of the stamens or the carpels. Maize plants initially produce both carpels and stamens in their tassel and ear flowers. During development, half of the carpels initiated in the ears and all the carpels initiated in tassel get suppressed and undergo programmed cell death. It however remains largely unknown which genes are implicated in carpel suppression and programmed cell death. *GRASSY TILLERS1 (GT1),* which encodes a class 1 HD-ZIP transcription factor is one of the few developmental genes known to suppress carpels in maize flowers. In *gt1* mutants, the carpels are partially derepressed in tassel flowers, indicating the existence of other genes that act with *gt1* to regulate carpel suppression. To identify which genes these are, an EMS enhancer screen was conducted on *gt1* mutants which yielded double mutants with completely depressed carpels that we called the *rapunzel1* (*rzl1*) mutants. Here, we describe the *gt1; rzl1* mutant phenotype. *gt1; rzl1* double mutants have completely derepressed carpels and silks coming out of the tassels. The gene enhancing this *gt1* phenotype is, yet to be identified, but preliminary phenotyping shows that *rzl1* acts with *gt1* to regulate both carpel and stamen development. The data further suggest that *rzl1* has different effects on upper vs. lower floret development in the maize tassel spikelet. The ears are, however, normal. These results show that the *rzl1* gene plays a role in sex determination and carpel suppression in maize plants and may have implications for plant growth and yield.

Funding acknowledgement: National Science Foundation (NSF)

P28 @tmtbiotech

Identification of candidate callus regenerative genes using genetic mapping and transcriptional profiling

(submitted by Tej Man Tamang <tejman@ksu.edu>)
Full Author List: Tamang, Tej Man[1]; Lin, Guifang[1]; Liu, Yan[2]; Park, Sunghun[3]; White, Frank F.[4]; Wei, Hairong[5]; Cho, Myeong-Je[6]; Liu, Yunjun[2]; Liu, Sanzhen[1]
[1] Department of Plant Pathology, Kansas State University, Manhattan, KS, USA 66506
[2] Institute of Crop Sciences, Chinese Academy of Agricultural Sciences, Beijing, China 100081
[3] Department of Horticulture and Natural Resources, Kansas State University, Manhattan, KS, USA 66506
[4] Department of Plant Pathology, University of Florida, Gainesville, FL, USA 32611
[5] College of Forest Resources and Environmental Science, Michigan Technological University, Houghton, MI, USA 49931
[6] Innovative Genomics Institute, University of California, Berkeley, CA, USA 94704

Callus formation, somatic embryogenesis, and plant regeneration play critical roles in maize genome engineering. Identification of underlying genes that drive these processes will improve maize genome engineering. Here, we performed bulk segregant RNA-seq (BSR-seq) and Genotyping-By-Sequencing (GBS) to analyze type I and type II calli that were generated from segregating progenies of a transformation-amenable line A188 and the transformation-recalcitrant inbred line B73. Five QTL loci in chromosomes 2, 5, 6, 8, and 9 were identified and explained 50.9% of the phenotypic variation. We also dissected fast growing and slow growing tissues within A188 calli and performed RNA-seq analysis. Gene Ontology (GO) term enrichment analysis of differentially expressed genes (DEGs) between callus types indicated that the DEGs were enriched in pathways related to transmembrane components, DNA binding, and cell wall organization. A hierarchical gene regulation networks was constructed to identify regulators in the callus formation process. Integration transcriptomic data with genetic mapping data and network analysis identified 39 candidate genes. Currently, validation tests on these candidate genes are being conducted using ectopic expression and CRISPR-Cas9 knockouts. This research will lead to improvements in the transformation and gene editing in maize.

Funding acknowledgement: National Science Foundation (NSF)

P29

Improved maize transformation service by Crop Bioengineering Laboratory at Iowa State University

(submitted by Jessica Ji <jjessica@iastate.edu>)
Full Author List: Ji, Jessica[1]; Grosic, Sehiza[1]; Lee, Keunsub[1 2]; Kang, Minjeong[2]; Whitham, Steven[1 3]; Wang, Kan[1 2]
[1] Crop Bioengineering Center, Iowa State University, Ames, IA 50011
[2] Department of Agronomy, Iowa State University, Ames, IA 50011
[3] Department of Plant Pathology/Entomology/Microbiology, Iowa State University, Ames, IA 50011

Iowa State University established the Crop Bioengineering Laboratory (CBL) in 2022 to meet the demand for plant transformation services in the research community. Its mission is to conduct research and provide expertise and services in crop genetic transformation, including improving transformation technologies and teaching and training students and researchers in plant transformation. Currently, CBL is well-positioned to provide fast turnaround and highly efficient transformation service for maize inbred B104 to both internal and external researchers. Utilizing an improved transformation protocol (Kang et al, 2022, Front Plant Sci. 2022 May 4;13:860971. doi: 10.3389/fpls.2022.860971) along with enhanced vector and plant selection systems, CBL is able to complete a maize transformation project within approximately two months from the date of initiation to the date of transplanting transgenic plantlets to the soil. The average transformation frequency is over 20%. CBL is ready to provide customers with quality products and superior services at competitive prices. Please contact Dr. Jessica Ji (jjessica@iastate.edu) for more information.

Funding acknowledgement: Vice President of Research Office at Iowa State University, College of Agriculture and Life Sciences at Iowa State University, College of Liberal Arts and Sciences at Iowa State University, Plant Science Institute at Iowa State University

P30

Improving altruistic transformation system for gene editing of public maize genotypes

(submitted by Minjeong Kang <mjkang@iastate.edu>)
Full Author List: Kang, Minjeong[1]; Lee, Keunsub[1]; Ji, Jessica[2]; Grosic, Sehiza[2]; Wang, Kan[1]
[1] Department of Agronomy, Iowa State University, Ames, IA 50011, USA
[2] Crop Bioengineering Laboratory, Iowa State University, Ames, IA 50011, USA

Although maize genetic transformation is routine for some public labs, only a limited number of maize genotypes can be readily transformable such as A188, Hi-II and B104. Recent advances in maize genetic transformation using morphogenic transcription factors (MTFs) allow efficient regeneration of recalcitrant genotypes. However, constitutive expression of MTFs often causes pleiotropic effects that inhibit the regeneration and development of explants. Altruistic transformation method (Hoerster et al., 2020) utilizes two separate T-DNA constructs. One construct contains a gene of interest (GOI), and the altruistic construct provides diffusible Wushel2 proteins, which can promote morphogenesis of neighboring cells without integration *Wuschel2* (*Wus2*) gene. This approach provides flexible options for gene editing and transgenesis approaches, for it can be readily combined with existing T-DNA vectors and does not require complex T-DNA vector construction which incorporates both MTFs and GOIs into a single T-DNA.Here we described the altruistic transformation system using public maize inbred lines B104, B73, TZI8, and CML277. An altruistic construct pKL2391 carries maize *Wus2* and a betalain biosynthesis gene cassette *RUBY* as a visual marker. A CRISPR-Cas9 construct, pKL2359, targeting *Glossy2* gene was used for gene editing. While pKL2359 was delivered by LBA4404Thy- strain, pKL2391 was delivered by one of the three auxotrophic *Agrobacterium* strains, LBA4404Thy-, EHA105Thy-, and EHA105TR (Thy-, *thyA* deficient; TR, *thyA,* and *recA* deficient). All *Agrobacterium* strains carried a ternary helper plasmid pKL2299 to enhance T-DNA delivery. In B104, two altruistic strains (LBA4404Thy- and EHA105TR) significantly increased the transformation frequencies compared to the control without altruistic strains ($P < 0.01$, two proportions z-test). For B73, TZI8, and CML277, both CRISPR-Cas9 and altruistic constructs were delivered by LBA4404Thy- strain. Altruistic transformation yielded higher regeneration of immature shoots from all three genotypes than the control without altruistic strain. Our preliminary results suggest that the altruistic transformation using pKL2391 could increase transformation frequency in B104, and this system could be applied to gene editing and the transformation of recalcitrant genotypes.

Funding acknowledgement: National Science Foundation (NSF)

P31

Integrating ethylene signaling into maize domestication regulatory pathways

(submitted by Josh Strable <jjstrabl@ncsu.edu>)
Full Author List: Strable, Josh[1]; Aragón-Raygoza, Alejandro[1]
[1] Department of Molecular and Structural Biochemistry, North Carolina State University

Plants are shaped largely by branching events. Branches grow from axillary buds, which are sensitive to the environment and subject to complex genetic regulation. Many domesticated crops have been bred for suppression of bud growth and subsequent reduced branching to improve performance under high density planting. During maize domestication, selection of a gain-of-function allele of *TEOSINTE BRANCHED1* (*TB1*) led to such increased apical dominance. Plant hormone pathways also have a key role in determining shoot architecture, some of which function downstream of *TB1*. The hormone ethylene acts largely as a growth inhibitor, often in the context of responding to environmental inputs. However, to our knowledge, a role for the ethylene signaling in maize shoot branching, or its potential relationship with domestication, has yet to be established. We recently discovered that mutations in maize *ETHYLENE INSENSITIVE3(EIN3)-LIKE1* (*ZmEIL1*) and *ZmEIL9* genes result in aerial lateral branch and inflorescence phenotypes that resemble maize domestication pathway mutants. Available RNA- and ChIP-seq data suggest that *ZmEIL* genes are putatively regulated by TB1. To characterize ethylene transcriptional response, we utilized single-cell transcriptomics of shoot apices from *Zmeil* and normal shoots with and without the ethylene precursor ACC. Many genes that are putatively downstream of *ZmEIL* are also differentially expressed in *tb1* tiller buds. These observations suggest that *ZmEIL* genes regulate important aspects of plant growth and support our working hypothesis that ethylene signaling has a previously undescribed role in domestication pathways that shape shoot architecture.

Funding acknowledgement: NCSU Start-up Funds

P32

Investigating the role of RAMOSA3 (RA3) in grass flower development and evolution

(submitted by Terice Kelly <tmkelly@umass.edu>)
Full Author List: Kelly, Terice[1]; de Neve, Amber[1]; Bartlett, Madelaine[1]
[1] Univeristy of Massachusetts Amherst, Amherst, Ma, 01003

Floral morphology is diverse in the grass family. This diversity is, in part, caused by organ suppression. Floral organ suppression is a vital process in floral development. For example, maize flowers initiate stamens and carpels, but unisexual flowers develop through organ suppression in both tassels and ears. RAMOSA3 (RA3) and GRASSY TILLERS1 (GT1) regulate carpel suppression in maize to create unisexual, staminate tassel flowers. The gt1; ra3 double mutant also has enhanced tillering. This phenotype suggests that GT1 and RA3 act together to regulate axillary meristem suppression. GT1, a transcription factor gene, regulates tiller growth downstream of another transcription factor, TEOSINTE BRANCHED1 (TB1). Here, we aimed to discover whether there was a genetic interaction between tb1 and ra3, as there is between gt1 and tb1, and between gt1 and ra3. We report on our mutant analyses investigating the genetic interactions between gt1, ra3, and tb1 in carpel suppression, tillering, and inflorescence branching. In addition, we aim to determine if RA3 has a conserved role in controlling floral organ suppression and inflorescence branching in the grasses. To do this, we are investigating the role of an RA3 homolog in Brachypodium distachyon, using CRISPR-Cas9 genome editing and publically available mutants. Our findings will reveal which genes are responsible for sculpting floral organs.

Gene / Gene Models described: *TB1, GT1, RA3*;
Zm00001eb054440,Zm00001eb007950,Zm00001eb327910,Bradi1g21420
Funding acknowledgement: National Science Foundation (NSF)

P33

KATANIN is required for microtubule severing, growth and division positioning in maize

(submitted by Stephanie Martinez <smart046@ucr.edu>)
Full Author List: Martinez, Stephanie E.[1]; Allsman, Lindy A.[1]; Lau, Kin H.[2]; Wright, Amanda J.[3]; Weil, Clifford[4 5]; Rasmussen, Carolyn G.[1]
[1] Department of Botany and Plant Sciences, Center for Plant Cell Biology, Institute for Integrative Genome Biology 900 E University Ave. 3126 Genomics, University of California Riverside, CA 92521
[2] Bioinformatics and Biostatistics Core, Van Andel Institute, Grand Rapids, MI 49503, USA
[3] Unaffiliated, Lewisville, TX, USA
[4] Purdue University, West Lafayette, IN 47907, USA
[5] Division of Molecular and Cellular Biosciences, National Science Foundation, Alexandria, Virginia 22314, USA

Microtubule dynamics and organization influence cell shape and cell division plane orientation. One protein complex involved in this process is KATANIN, a microtubule severing AAA ATPase protein complex composed of a catalytic p60 and a regulatory WD-40-containing p80 subunit. The p60 catalytic subunit forms hexamers and is sufficient to sever microtubules through ATP hydrolysis. However, binding of the KATANIN protein complex to microtubule severing sites is mediated by the p80 subunit, allowing for higher severing efficiency of microtubules. In 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). dcd3a-2 dcd3b contains mutations that disrupt the oligomerization domain of KATANIN, which may impede functional hexamer formation. The Clt1 mutant contains a missense mutation in the ATPase domain, which may disrupt functional ATP-hydrolysis and microtubule severing. To determine how these mutations influence KATANIN's microtubule severing function, microtubule severing was assessed in various mutants. Thus far, loss-of-function dcd3a-2 dcd3b mutants have reduced severing. dcd3a-2 or dcd3b single mutants also have reduced microtubule severing when compared to wild type, but not as severe as dcd3a-2 dcd3b, suggesting both functional copies of the oligomerization domain of KATANIN are required for proper severing ability in maize. Clt1 heterozygous mutants have no significant difference in microtubule severing in elongating cells when compared to wild type. In vitro ATPase activity assays of mutant and wild-type proteins will be used to characterize ATPase activity of KATANIN p60 subunits. Characterizing KATANIN function in maize will lead to 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)

P34

Liguleless narrow and *narrow odd dwarf* act in overlapping pathways to regulate maize development and metabolism

(submitted by Jacob Brunkard <brunkard@wisc.edu>)
Full Author List: Brunkard, Jacob O.[1]; Abraham-Juarez, M. Jazmin[2]; Busche, Michael[1]; Anderson, Alyssa A.[3 4]; Lunde, China[4]; Winders, Jeremy[5]; Christensen, Shawn A.[6]; Hunter, Charles T.[7]; Hake, Sarah[3 4]
[1] Laboratory of Genetics, University of Wisconsin, Madison, Wisconsin 53706, USA
[2] Laboratorio Nacional de Genomica para la Biodiversidad, Unidad de Genomica Avanzada, Centro de Investigacion y de Estudios Avanzados del Instituto Politecnico Nacional, Guanajuato 36821, Mexico
[3] Department of Plant and Microbial Biology, University of California, Berkeley, California 94720, USA
[4] Plant Gene Expression Center, USDA Agricultural Research Service, Albany, California 94710, USA,
[5] Genomics and Bioinformatics Research Unit, US Department of Agriculture-Agricultural Research Service, Raleigh, NC, USA
[6] Nutrition, Dietetics, and Food Science, Brigham Young University, Provo, Utah 84602, USA
[7] Chemistry Research Unit, USDA Agricultural Research Service, Gainesville, Florida 32608, USA

Narrow odd dwarf (nod) and *Liguleless narrow* (Lgn) are pleiotropic maize mutants that both encode plasma membrane proteins, cause similar developmental patterning defects, and constitutively induce stress signaling pathways. To investigate how these mutants coordinate maize development and physiology, we screened for protein interactors of NOD by affinity purification. LGN was identified by this screen as a strong candidate interactor, and we confirmed the NOD-LGN molecular interaction through orthogonal experiments. We further demonstrated that LGN, a receptor-like kinase, can phosphorylate NOD *in vitro*, hinting that they could act in intersecting signal transduction pathways. To test this hypothesis, we generated *Lgn-R;nod* mutants in two backgrounds (B73 and A619), and found that these mutations enhance each other, causing more severe developmental defects than either single mutation on its own, with phenotypes including very narrow leaves, increased tillering, and failure of the main shoot. Transcriptomic and metabolomic analyses of the single and double mutants in the two genetic backgrounds revealed widespread induction of pathogen defense genes and a shift in resource allocation away from primary metabolism in favor of specialized metabolism. These effects were similar in each single mutant and heightened in the double mutant, leading us to conclude that NOD and LGN act cumulatively in overlapping signaling pathways to coordinate growth-defense tradeoffs in maize.

Gene / Gene Models described: *lgn1, nod1*; Zm00001eb382080, Zm00001eb004320
Funding acknowledgement: National Institutes of Health (NIH), National Science Foundation (NSF), United States Department of Agriculture (USDA), UC-Mexus CONACYT

P35 @thanduanlung

Linking brace root development to function

(submitted by Thanduanlung Kamei <thanduan@udel.edu>)
Full Author List: Kamei, Thanduanlung[1]; Chuck, George[2]; Sparks, Erin E.[1]
[1] Department of Plant and Soil Sciences, University of Delaware, Newark, DE. 19716, USA.
[2] Department of Plant & Microbial Biology, University of California-Berkeley, Berkeley, CA. 47201, USA

Root systems play an important role in water and nutrient uptake, microbial interactions, and crop anchorage. Anchorage is especially important given the increasing prevalence and severity of storm systems. In maize, it has been shown that the roots that originate from nodes above the soil (called brace roots) are especially important in anchorage and lodging resistance. However, it is unknown how the molecular regulation of brace root development may be used to modulate anchorage. Previous work in maize has identified a link between nodal root development and the juvenile-to-adult transition, however, it was unknown if this link was direct or indirect. We performed RNA-sequencing of stem nodes during different stages of brace root development and show that SBP transcription factors, known regulators of the juvenile-to-adult transition, are directly expressed in brace root developing nodes. Thus, we hypothesize that SBP expression would affect nodal root formation and have functional consequences. To test this hypothesis, we quantified anchorage in the dominant *Corngrass1* (*Cg1*) mutants a miR156 overexpression line, which targets the *SBP/SPL* gene family, and individual sbp mutants (ub2, ub3, tsh4). Through this analysis, we identified sbp mutants have more whorls of brace roots entering the soil and an increased root torsional stiffness compared to their wildtype control. Together these data support the proposition that modulating brace root development can be used to alter anchorage.

Funding acknowledgement: National Science Foundation (NSF)

P36
Maize Kernel Grafting: A simplified method

(submitted by David Zimmerman <djz3@illinois.edu>)
Full Author List: Zimmerman, David J.[1]; Studer, Anthony J.[1]
[1] Department of Crop Sciences, University of Illinois Urbana-Champaign, Urbana, IL 61801

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 has reported a method by which it is possible to graft both inter and intra-specific monocot species by performing the grafting in pre-germinated seeds and kernels. Building on this work, we present the results from a simplified grafting method that uses a single transverse cut through the mesocotyl of an ungerminated maize kernel. The embryos of the bisected kernels are then re-aligned, held together with a small amount of adhesive, and allowed to germinate as normal. A modified CFDA dye assay and genotyping with molecular markers were used to confirm the vascular reconnection of the scion and rootstock tissues. The method offers additional evidence that *Zea mays* is a graft compatible species and that grafted maize plants can grow successfully to maturity. We expect that with its ease of use and rate of success, this simplified method will become the standard grafting protocol for maize in the future. Grafting in maize will allow researchers to unravel long-distance signaling networks and secondary metabolite production as well as offering a potential platform for novel gene editing techniques.

Funding acknowledgement: National Science Foundation (NSF), CROPPS

P37
Maize developmental transcription factors that regulate leaf initiation, morphology, and phyllotaxy

(submitted by Lander Geadelmann <landerg@iastate.edu>)
Full Author List: Geadelmann, Lander[1]; Unger-Wallace, Erica[1]; Strable, Josh[2]; Vollbrecht, Erik[1]
[1] Iowa State University; Department of Genetics, Cellular, and Developmental Biology; Ames, IA
[2] North Carolina State University; Department of Molecular and Structural Biochemistry; Raleigh, NC

The regular pattern of leaf initiation at the shoot apical meristem (SAM) determines a plant's arrangement of leaves around a stem, or phyllotaxy. In maize phyllotaxy, leaves are initiated on alternating sides of the plant. Among all plants, few genes have been identified that influence phyllotaxy. In maize, mutants for the *aberrant phyllotaxy1* (*abph1*) cytokinin signaling gene have altered phyllotaxy such that leaves are initiated on opposite sides of the plant in pairs. *abph1* mutants have an enlarged SAM which is proposed to result from complex interactions between the hormones cytokinin and auxin. Penetrance and expressivity of the *abph1* mutant is dramatically reduced when converged into standard inbred lines. Our lab has identified the transcription factors *ereb130, ereb184*, and related paralogs as additional mutants that result in irregular leaf initiation and altered phyllotaxy reminiscent of *abph1*, with relatively high penetrance in standard inbred lines. Meristem size is unaffected in the single mutants examined, and expression analysis of *ereb130* mutants indicates a potential reduction in the biosynthesis of auxin, a hormone necessary for leaf initiation. Double mutants for *ereb130* and *abph1* act synergistically to dramatically alter phyllotaxy throughout the shoot and increase total number of leaves a plant produces. These results support the hypothesis involving cytokinin-auxin interaction in phyllotaxy, which we are investigating using fluorescent auxin and cytokinin reporters in the SAM. In addition to altered leaf initiation, *ereb130* mutants exhibit pleiotropic effects on internode length and on leaf morphology, with longer and wider leaves compared to wild type. The alteration of these traits is exacerbated by mutating paralogs and is dependent upon the allele and allelic combinations of genes, as well as the genetic (inbred) background, as determined by crossing existing mutants in multiple genetic backgrounds and generating new alleles through *Ds* transposon remobilization and CRISPR-Cas9 mediated genome editing.

Gene / Gene Models described: *abph1, ereb130, ereb184*; Zm00001eb076960, Zm00001eb005740, Zm00001eb058850

Funding acknowledgement: National Science Foundation (NSF)

P38

Maize *Cpd6* encodes a sucrose synthase hypothesized to uniquely function in cell wall development in the phloem

(submitted by Jada Smith <jspmf@umsystem.edu>)
Full Author List: Smith, Jada[1]; Leach, Kristen[2]; McCubbin, Tyler[1]; Chomet, Paul[4]; Wagner, Ruth[4]; Grote, Karen[4]; Peevers, Jeanette[4]; Slewinski, Thomas[4]; Julius, Benjamin[4]; Jackson, Dave[3]; Lui, Lei[3]; Koch, Karen[2]; Lui, Peng[2]; Braun, David[1]
[1] University of Missouri, Columbia, MO, 65202
[2] University of Florida, Gainesville, FL 32611
[3] Cold Spring Harbor Laboratory
[4] Bayer Crop Science, Chesterfield, Missouri 63017, USA

Cellulose is Earth's most abundant biological polymer, as it compromises the major structural component of plant cell walls. Cellulose is synthesized by cellulose synthase, which catalyzes the polymerization of uridine diphosphate glucose (UDP-G) into cellulose. This complex but poorly understood process is crucial in cell wall biosynthesis. *Carbohydrate partitioning defective6* (*Cpd6)* encodes a sucrose synthase (SUSY) hypothesized to provide UDP-G to cellulose synthase as well as to callose synthase. SUSY proteins interconvert sucrose and UDP into fructose and UDP-G for plant metabolism. Surprisingly, in both wild-type and *cpd6* mutants, distantly related, well-characterized SUSY proteins immunolocalized to the leaf companion cells, where they are highly expressed. The presence of abundant SUSY proteins in the companion cells suggests that *cpd6* mutants have sufficient SUSY levels in the phloem but perplexingly are still defective in exporting sucrose. To characterize the partitioning of carbon to the cell walls, we investigated the abundance of callose and cellulose in *cpd6* mutants and wild-type controls. The findings presented will provide new insights into the biogenesis of plant cell walls, determine the role(s) for SUSY proteins in providing the cell wall polymers precursor, and will deepen our knowledge of how plants partition carbohydrate resources.

Funding acknowledgement: National Science Foundation (NSF)

P39

Mapping the maize TOR signaling network

(submitted by Michael Busche <busche2@wisc.edu>)
Full Author List: Busche, Michael[1]; Brunkard, Jacob[1]
[1] University of Wisconsin, Madison; Madison, WI USA 53703

TARGET OF RAPAMYCIN (TOR) is a conserved protein kinase that coordinates eukaryotic growth and metabolism with nutrient availability. TOR has been well-studied in biomedical contexts due to its central roles in metabolic diseases and cancers but has only recently gained attention in plant biology. In an effort to explore TOR signaling in maize, I first developed a nutrient-limiting assay to precisely toggle TOR activity in developing maize embryos. Then, I performed global profiling experiments (e.g., transcriptomics and proteomics) using these embryos grown in TOR-activating and -inactivating conditions. Combined with phenotypic and targeted molecular analyses, these experiments have comprehensively mapped the maize TOR signaling network. This map revealed that key signaling axes are conserved in Arabidopsis and maize, while also identifying new players in TOR-dependent gene regulatory networks that are specific to maize. Long-term, I seek to understand how we can leverage the TOR signaling network to improve maize yields and resiliency.

Gene / Gene Models described: *tor1*; Zm00001eb285840

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

P40

Master Meristem Manipulators: Regulation of meristem size by the REL2 corepressor family

(submitted by Jason Gregory <jason.gregory@rutgers.edu>)
Full Author List: Gregory, Jason[1 2]; Liu, Xue[1]; Chen, Zongliang[1]; Gallavotti, Andrea[1 2]
[1] Waksman Institute of Microbiology, Rutgers University, Piscataway, New Jersey 08854-8020, USA
[2] Department of Plant Biology, Rutgers University, New Brunswick, New Jersey USA 08901, USA

In plants, growth of the body throughout the life cycle occurs post-embryonically through the action of meristems. Apical meristems form the vegetative shoot and root systems, whereas axillary meristems form branches and reproductive structures. Prior characterization of *ramosa1 enhancer locus2* (*rel2)* mutants revealed pleiotropic vegetative and reproductive phenotypes such as defective axillary meristem initiation and inflorescence meristem (IM) maintenance in single recessive mutants (PMID:30348817). In maize, the *REL2* family of transcriptional corepressor proteins is comprised of four members, *REL2*, *REL2-LIKE1 (RELK1), REL2-LIKE2 (RELK2*), and *REL2-LIKE3 (RELK3*). *rel2;relk1* double mutants were identified in an enhancer screen of the *rel2-ref* mutant allele, whereas loss-of-function mutations in *RELK2* and *RELK3*, two paralogous genes co-orthologous to Arabidopsis *TOPLESS* (*TPL*), were generated by CRISPR-Cas9. Interestingly, *rel2;relk1* mutants had enhanced ear fasciation (among other phenotypes). Conversely, triple *rel2;relk2;relk3* mutants revealed a shoot apical meristem termination phenotype. Triple mutant seeds either failed to germinate or developed few leaves before apical growth termination. We are currently performing *in-situ* hybridization with different marker genes to confirm loss of meristematic identity in SAM remnants of triple *rel2;relk2;relk3* mutants. By sectioning developing embryos 10 days after pollination (DAP), 14 DAP, and 21 DAP we determined that defective SAM maintenance in higher order mutants begins during early embryogenesis. Recently, *rel2;relk2* and *rel2;relk3* double mutants were also generated to explore functional redundancy among distinct family members. We report that while *rel2;relk2* and *rel2;relk3* double mutants had tassel defects similar to *rel2;relk1*, *rel2;relk3* double mutants were predominantly earless while *rel2;relk2* double mutant ears were fasciated, similar to *rel2;relk1* plants. This suggests that while *RELK2* and *RELK3* are functionally homologous to *TPL*, as is *REL2* (PMID:20699296),*RELK*s may have both a partially divergent and redundant function in meristem regulation together with *REL2*.We acknowledge funding from the National Science Foundation (IOS#2026561).

Gene / Gene Models described: *REL2, RELK1, RELK2, RELK3*; Zm00001eb415530, Zm00001eb127680, Zm00001eb011010, Zm00001eb398420

Funding acknowledgement: National Science Foundation (NSF)

P41

Molecular regulation for improving maize androgenesis

(submitted by Xia Zhang <xia.zhang@syngenta.com>)
Full Author List: Zhang, Xia[1]; Burch, Erin[1]; Egger, Rachel L.[1]; Green, Julie[1]; Gu, Weining[1]; Liu, Weiguo[1]
[1] Seeds Research, Syngenta Crop Protection, Research Triangle Park, North Carolina, United States

Androgenesis, a developmental program that switches haploid microspores from their normal pollen development towards an embryogenic pathway, represents an important tool for creating double haploid (DH) lines therefore accelerating plant breeding. In maize, haploid induction from isolated microspores has remained low efficiency and is highly dependent on the genotype. Past research on androgenesis has focused extensively on tissue culture-based protocol development with little progress in maize. Our research sought to improve androgenesis induction and embryo formation using molecular approach to target cellular pathways important for androgenic embryo development. Our results indicate that manipulating starch biosynthetic gene expression through gene editing and gene silencing, accompanied by a modified microspore culture protocol, can trigger microspore reprogramming and drive a substantial increase in embryo induction. These results are the first demonstration of the potential of molecular regulation strategies to enable microspore embryogenesis.

P42

Monothiol glutaredoxin AtGRXS17-expressing maize plants increase yield under combined heat and drought stress

(submitted by Gergely Motolai <gmotolai@ksu.edu>)
Full Author List: Motolai, Gergely[1]; Tamang, Tej M.[1]; Stewart, Nathan F.[1]; Avalos-Calleros, Jesús A.[1]; White, Frank F.[2]; Liu, Sanzhen[3]; Park, Sunghun[1]
[1] Department of Horticulture and Natural Resources, Kansas State University, Manhattan, KS, 66506
[2] Department of Plant Pathology, University of Florida, Gainesville, FL 32611
[3] Department of Plant Pathology, Kansas State University, Manhattan, KS, 66506, USA

Combined heat and drought stress, which are linked to climate change and global warming, have spanned continents in recent years, challenging global maize production. The development of maize cultivars with enhanced tolerance to combined heat and drought stress, particularly during the reproductive stage, has the potential to greatly impact the production of maize worldwide. Here, we report that ectopic expression of Arabidopsis monothiol glutaredoxin, AtGRXS17, improved maize yields under combined heat and drought stress. A 3-day combined heat and drought stress was imposed on wild-type and AtGRS17-expressing maize lines at silking stage, and the subsequent analysis found that expression of AtGRXS17 resulted in a two-fold increase in grain production as compared to wild-type plants. Stomatal conductance analysis showed that stomatal closure occurred early in wild-type plants. Further, hydrogen peroxide content in wild-type plants dramatically increased after combined heat and drought stress, indicating that wild-type plants were highly stressed in comparison to AtGRS17-expressing maize lines. These results indicate that AtGRXS17 expression protects maize plants and improves yield under combined heat and drought stress.

Funding acknowledgement: Kansas Corn Commision

P43

Morphogenic genes assisted Agrobacterium-mediated transformation of tropical maize genotypes

(submitted by Mercy Azanu <mkazanu@iastate.edu>)
Full Author List: Azanu, Mercy K.[1]; Kang, Minjeong[1]; Ghenov, Fernanda[2]; Keunsub, Lee[1 3]; Muszynski, Michael[2]; Wang, Kan[1 3]
[1] Department of Agronomy, Iowa State University, Ames, IA, USA
[2] Department of Tropical Plant and Soil Sciences, University of Hawai'i at Mānoa, Honolulu, HI, USA
[3] Crop Bioengineering Center, Iowa State University, Ames, Iowa, USA

Maize is one of the major cereal crops grown worldwide, requiring its broad adaptation to tropical or temperate growing environments. Tropical maize is photoperiod sensitive and flowers under short day conditions, whereas temperate maize is relatively insensitive and flowers under both short and long days. Although tropical and temperate maize have many desirable traits, their different flowering conditions make it nearly impossible to use them in the same breeding program. To address this, a robust transformation system must be established to enable efficient genome engineering to produce temperate adapted tropical maize lines with the ability to flower under long days. A major bottleneck to maize transformation is genotype and explant dependency. Recently, enhanced regeneration of recalcitrant genotypes using morphogenic transcription factors (MTFs) *Baby boom* (*BBM*) and Wuschel2 (*Wus2*) has been developed by scientists at Corteva Agriscience. Here, we report successful transformation of tropical maize lines Tzi8, CML277, CML258 and CML10, using *Agrobacterium*-mediated transformation methods. *Agrobacterium* strain LBA4404Thy-, helper plasmid (PHP71539), and several MTF constructs which were kindly provided by Dr. William Gordon-Kamm (Corteva Agriscience). Specific constructs were used for either immature embryo or leaf transformation, with *HRA* or *NPTII* as the selectable marker gene, respectively. MTF cassettes were removed before regeneration using different inducible promoters to express the *cre* gene. Using immature embryos, we achieved 1.4% quality events (transgenic plants with excised MTFs and without plasmid backbone) for inbred CML277. Leaf transformation was done using ~3 cm leaf whorl dissected from the base of two weeks old seedling shoots for infection. While final successful rates are not available currently, a 20% regeneration rate with an 18% excision rate were achieved for leaf transformation. Using seedling leaf whorls as explants removes the high cost of greenhouse maintenance and challenges associated with the production of high quality immature embryos year-round for transformation.

Funding acknowledgement: National Science Foundation (NSF)

P44 @_DianaRuggiero

Quantitative genetics and high-throughput phenotyping of maize leaf vascular traits

(submitted by Diana Ruggiero <ruggiedi@oregonstate.edu>)
Full Author List: Ruggiero, Diana[1]; Dao, Thai[1]; Chuck, George[2]; Leiboff, Samuel[1]
[1] Oregon State University, Corvallis, Oregon, 97331
[2] UC Berkeley, Berkeley, California, 94720

Maize tissues that engage in efficient C_4 photosynthesis have high vein density in order to maintain the requisite proximity between mesophyll and bundle sheath cells. Vascular density varies across the sheath, auricle, and blade compartments of the leaf. Although mature leaf veins are anatomically similar, in maize and other C_4 grasses there are several different vein types occurring in a stereotypical developmental sequence and spatial configuration throughout the sheath, auricle and blade. Despite the need for consistent cellular spacing between veins, vascular traits are surprisingly variable between genotypes. For example, instances of defective spacing result in 'merged' veins, where there are no mesophyll cells between the bundle sheath cells of two neighboring veins. An increased number of merged veins appear in many genotypes and on a genetically consistent basis. This trait has been previously associated with mutations in genes scarecrow (*scr)* and shortroot (*shr*). We here demonstrate that the merged vein trait is additionally present in many inbred lines with varying severity. To screen for natural variants of vascular development programs, we are furthermore conducting a GWAS correlating vein density with markers from the Wisconsin Diversity Panel (WiDiv). Over the course of two field seasons, we have collected over 5000 leaf samples from more than 750 different maize lines. To facilitate this large-scale study of microscopic traits, we have devised a deep-learning based automated phenotyping system for estimating type-specific vein density in cleared leaf images. This system employs an implementation of U-Net, a convolutional neural network (CNN) architecture for semantic segmentation. This system classifies and masks the various vein types in each sample (including instances of aberrant merged veins), allowing for quantification of different vein subtypes and their relative distributions.

Funding acknowledgement: National Science Foundation (NSF)

P45

RAMOSA3 interacts with catalytic and non-catalytic TREHALOSE-6-PHOSPHATE SYNTHASES (ZmTPSs) to control embryo and inflorescence development

(submitted by Thu Tran <tran@cshl.edu>)
Full Author List: Tran, Thu M[1]; Claeys, Hannes[2]; Michalski, Kevin[1]; Boumpas, Panagiotis[4]; Vi, Son L[3]; Furukawa, Hiro[1]; Jackson, David[1]
[1] Cold Spring Harbor Laboratory, 1 Bungtown Road, Cold Spring Harbor, NY11724, USA
[2] Inari, Industriepark 7A, 9052 Zwijnaarde, Belgium
[3] Faculty of Biotechnology, Chemistry and Environmental Engineering, PHENIKAA University, Yen Nghia, Ha Dong, Hanoi 12116, Vietnam
[4] Heidelberg University, Centre for Organismal Studies (COS) Heidelberg, Department of Developmental Biology, Heidelberg, Germany

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. T6P has emerged as an important regulator of sugar metabolism, stress responses, and cell fate decisions; however, the underlying mechanisms remain elusive. In maize, the classical mutant *ramosa3* (*ra3*) increases inflorescence branching and RA3 encodes a catalytic TPP but its enzyme activity is not responsible for controlling branching. To further explore the molecular mechanism of RA3's "moonlighting" 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 might 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 (cryo-EM). 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.

Gene / Gene Models described: *ra3, tps1, tps12, tps11, tps14*; Zm00001d02219, Zm00001d028267, Zm00001d038728, Zm00001d020396, Zm00001d010755
Funding acknowledgement: National Science Foundation (NSF)

P46

Regulation and evolution of reproductive phasiRNAs in maize and related species

(submitted by Junpeng Zhan <jzhan@danforthcenter.org>)
Full Author List: Zhan, Junpeng[1]; O'Connor, Lily[1 2]; Marchant, D. Blaine[3]; Teng, Chong[1]; McGregor, Madison[1]; Lewis, Scott[1 4]; Walbot, Virginia[3]; Meyers, Blake C.[1 5]
[1] Donald Danforth Plant Science Center, St Louis, Missouri 63132, USA
[2] Department of Biology, Washington University, St Louis, Missouri 63130, USA
[3] Department of Biology, Stanford University, Stanford, California 94305, USA
[4] Division of Biology and Biomedical Sciences, Washington University, St Louis, Missouri 63130, USA
[5] Division of Plant Science and Technology, University of Missouri, Columbia, Missouri 65211, USA

The anther-enriched reproductive phasiRNAs play vital roles in sustaining male fertility in grass species. A few putative transcriptional regulators of the 21- or 24-nucleotide phasiRNA loci (referred to as *21-* or *24-PHAS* loci) have been identified in maize, but whether any of the transcription factors (TFs) suffice to activate any PHAS locus is unclear. We identified the temporal gene coexpression networks (modules) associated with maize anther development, including modules highly enriched for the *21-* or *24-PHAS* loci. *Trans*-activation assays in maize protoplasts of individual TFs using bulk-protoplast RNA-seq showed that two of the TFs coexpressed with *21-PHAS* loci could activate several 21-nt phasiRNA pathway genes but not transcription of *21-PHAS* loci. We also present evolutionary analyses of the phasiRNA pathways in maize and related species and functional characterization of Argonaute (AGO) proteins involved in the pathways.

Funding acknowledgement: National Science Foundation (NSF)

P47 @CraigCowling01

Roles of auxin transporter PILS6 in maize growth and development

(submitted by Craig Cowling <ccowling@iastate.edu>)
Full Author List: Cowling, Craig[1]; Houmayani, Arielle[2]; McReynolds, Maxwell[1]; Draves, Melissa[1]; Lang, Michelle[1]; Ke, Haiyan[3]; Dehesh, Katayoon[3]; Strader, Lucia[2]; Walley, Justin[1]; Kelley, Dior[1]
[1] Iowa State University; Ames, Iowa
[2] Duke University; Durham, North Carolina
[3] University of California Riverside; Riverside, California

The phytohormone auxin is essential for regulating plant growth and development. Auxin accumulation in meristems is required to maintain stem cell populations and auxin transport can facilitate cellular differentiation during organogenesis. Auxin transporters are required for maize shoot development but those that underpin maize root development are not known. Within the primary root, free indole-3-acetic acid (auxin) levels are asymmetrically distributed, suggesting that this pattern is established by regulated transport and/or biosynthesis. Using reverse genetics, we have identified two putative candidates in the PIN-LIKES (PILS) auxin efflux carrier family that are required for proper auxin transport *in vivo*. Loss of function transposon alleles of *PILS6* displays altered auxin response in seedlings and impacts multiple aspects of maize development, including shoot height and crown root architecture. Transient expression of fluorescently tagged PILS6 protein shows it is localized to the endoplasmic reticulum. Expression of PILS6 in yeast demonstrates that it is a bona fide transporter of auxin. Loss of PILS6 leads to extensive remodeling of the proteome and phosphoproteome compared to W22. A co-expression network was reconstructed using these expression data to identify potential protein partners that may act in concert with PILS6. Based on these findings I will present a working model for PILS6 in regulating root and shoot formation, which may inform strategies to generate desirable architecture traits.

Gene / Gene Models described: *pils6*; Zm00001d043083
Funding acknowledgement: United States Department of Agriculture (USDA)

P48 @ErinSparksPhD

SMURF: A new tool to measure root torsional stiffness for understanding root lodging-resistance

(submitted by Erin Sparks <esparks@udel.edu>)
Full Author List: Hostetler, Ashley N[1]; Reneau, Jonathan W[1]; Sparks, Erin E[1]
[1] Department of Plant and Soil Sciences, University of Delaware, Newark, DE, USA 19713

Mechanical failure, known as lodging, has dramatic impacts on the quality and quantity of maize yields. This failure can occur at stalks (stalk lodging or greensnap) or at roots (root lodging). For root failure, assessing the risk for lodging and developing targeted improvement strategies has lagged due to a lack of reliable metrics associated with lodging risk. We have previously shown that the root system acts as a rotation spring, and thus designed a tool to measure the rotational stiffness of this spring with the hypothesis that the root torsional stiffness is directly related to root lodging. Our new tool, named SMURF (Sorghum & Maize Under Rotational Force), non-destructively quantifies root torsional stiffness in the field. To validate this tool, we measured the root torsional stiffness of 11 maize hybrids with variable susceptibility to root lodging over multiple years and planting densities. Increasing planting density reduces plant size, and we see an associated reduction in root torsional stiffness. However, when assessing the relationship between root torsional stiffness and root lodging within a given density, we find that genotypes with a higher root torsional stiffness have greater lodging. While it is perhaps counterintuitive that stiffer root systems are prone to failure, this result is consistent with earthquake engineering principles. Thus, the ideal root system must be strong enough to support the plant, but flexible enough to absorb external forces. These results demonstrate the utility of the SMURF tool for providing a quantitative, non-destructive assessment of root anchorage.

Funding acknowledgement: National Science Foundation (NSF)

P49

Screening maize orthologous genes in *Arabidopsis thaliana* for defects in moisture-regulated root branching

(submitted by Taylor Clarke <tlclarke@stanford.edu>)
Full Author List: Clarke, Taylor L.[1]; Scharwies, Johannes D.[1]; Dinneny, José R.[1]
[1] Stanford University; Stanford, CA, USA 94305

Lateral root branching is an important process in the development of a plant in order to optimize water and nutrient uptake. Hydropatterning is a type of lateral root branching response to local moisture availability. This moisture-regulated response promotes the development of lateral root primordia to grow in moist conditions and prevent development in dry conditions, so as to optimize resources. The genes integral to this process are not yet well understood and a comparison between multiple species could illuminate whether hydropatterning is conserved and possibly an advantageous trait. Maize is an ideal model to study hydropatterning due to high lateral root density, diverse germplasm availability, and good genetic resources. In an earlier study, hydropatterning was characterized in 248 diverse maize inbred lines. Primary roots of maize seedlings were grown with one side exposed to moist germination paper, the other exposed to air. We define strong hydropatterning as having all lateral root growth directed towards the moist paper and weak hydropatterning having all lateral root growth directed towards the air side. Genome/Transcriptome Wide Association Studies were performed to identify hydropatterning-associated loci and genes. To quickly follow up candidate genes, we used orthologous T-DNA lines in *Arabidopsis thaliana* in a root growth screen on Gelzan plates. Despite evolutionary differences between maize and Arabidopsis, we observed defects in hydropatterning as well as other architectural changes such as increases in the number and density of lateral roots, and changes to primary root length. Further investigation will identify the function of these genes in hydropatterning and root development.

Funding acknowledgement: Department of Energy (DOE)

P50

Single cell RNA-seq and confocal imaging analyses of blade-sheath boundary development in the maize leaf.

(submitted by Lukas Evans <Le95@cornell.edu>)
Full Author List: Evans, Lukas J.[1]; Fitzgerald, Elizabeth A.[1]; Wu, Hao[1]; Sylvester, Anne W.[2]; Scanlon, Michael J.[1]
[1] Cornell University, Ithaca, NY 14850
[2] Marine Biological Laboratory, Woods Hole, MA 02543

The blade-sheath boundary (BSB) of the maize leaf comprises two distinct tissues, an epidermally-derived ligule and a hinge-like auricle, that delineate the transition from distal blade to proximal sheath identity. Ligule and auricle develop on young leaf primordia, before blade and sheath identity are morphologically distinguished. Although transcriptional analyses have been conducted on initiating maize ligules, the genetic mechanisms of later stages in ligule outgrowth are unknown. Likewise, although the auricle contributes to leaf angle, an agronomically important trait, auricle ontogeny is poorly characterized and auricle-specific genetic markers are not identified. 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. Previous work used tissue-specific transcriptomics to compare ligule initiation in wild-type and *lg1*leaf primordia. Here, we utilize single cell transcriptomics to examine multiple stages of ligule/auricle outgrowth and patterning. Uniform Manifold Approximation and Projection (UMAP) analyses identified nineteen distinct cell clusters from the early-fringe stage of ligule/auricle development; two distinct cell clusters are identified as ligule- and auricle-specific. Differentially expressed genes in these ligule and auricle cell clusters suggest novel marker gene candidates. In addition, the coordinated development of the auricle during specific stages of ligule outgrowth was analyzed via confocal imaging of fluorescent markers of cell division. We found that at the early-fringe stage of ligule development, the auricle comprises a thin rectangular band of small irregularly shaped cells spanning the abaxial surface of the leaf. Subsequently, this narrow band of cells becomes thicker via mediolaterally-asymmetrical transverse anticlinal divisions, and eventually forms a triangular wedge shape by the late-fringe stage of ligule development. Interestingly, although stomata are not found in the auricle, stomatal cells are arranged as linear, coordinated cell files in the distal blade and proximal sheath, interrupted by the intervening auricle.

Gene / Gene Models described: *lg1*; GRMZM2G036297

Funding acknowledgement: National Science Foundation (NSF)

P51

Single-cell analysis of plant shoot meristems opens a 'goldmine' for functional studies

(submitted by Xiaosa Xu <xxu@cshl.edu>)
Full Author List: Xu, Xiaosa[1]; Passalacqua, Michael[1]; Gillis, Jesse[1 2]; Jackson, David[1]
[1] Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724, USA
[2] University of Toronto, Toronto, Ontario, M5S 3E1, CANADA

Shoot meristems determine plant architecture and impact crop productivity. An understanding of these structures requires insights into developmental domains, and the gene networks required to specify them. However, these domains are classified mainly by morphology, or insights from classical genetics, but this knowledge is limited by genetic redundancy and pleiotropy. Here, we constructed a single-cell gene expression atlas of maize inflorescence shoot meristems. We revealed novel developmental markers and validated them by bulk RNA-seq and mRNA *in situ*. Next, we created a gene co-expression network at single-cell resolution to predict genetic redundancy. We also integrated transcription factors ChIP-Seq with single-cell data to build transcriptional regulatory networks. Finally, we combined Genome-Wide Association Studies with single-cell data to identify yield-associated genes. We further successfully captured rare stem cells in *Arabidopsis* and maize shoot meristems that were largely missed in previous plant shoot single-cell studies. We identified stem cell markers and validated their expression using a high-resolution spatial analysis approach. We conducted a cross-species single-cell analysis to discover conserved stem cell markers and cell types. Plant stem cells are maintained by a conserved CLAVATA-WUSCHEL (CLV-WUS) pathway. We thus also profiled single cells from the inflorescence shoot apices of mutants of crucial regulators in CLV-WUS pathway. We found hundreds of differential expressed genes (DEGs) in the stem cells by comparing these mutants with wild type. We used multiplex CRISPR/Cas9 to knock out selected DEGs in a family of predicted sugar kinases, and found a striking meristem termination phenotype, validating the predictive power of our single-cell atlas. Together, this comprehensive shoot meristem single-cell atlas will open a 'goldmine' for functional studies at a fundamentally new level, and will be a valuable resource for the plant community.

Funding acknowledgement: National Science Foundation (NSF)

P52

Spatial transcriptomic analysis of the maize embryo

(submitted by Hao Wu <hw388@cornell.edu>)
Full Author List: Wu, Hao[1]; Scanlon, Michael J.[1]
[1] School of Integrative Plant Science, Cornell University, Ithaca, NY 14853

Maize embryo development initiates the histological and morphogenetic patterns that are reiterated throughout shoot development. Diverse cell types, tissues, and organs are formed in a dynamic, spatial-temporal manner. Stage 1 is strategic for studies of embryo development, as the earliest timepoint when all types of lateral organs are formed (i.e. the scutellum, coleoptile and leaf). We are interested in determining the homology of the grass cotyledon and its relationship to foliar leaves. In this study we used laser microdissection RNAseq (LM-RNAseq), single-cell RNAseq (scRNA-seq), and spatial transcriptomics to profile the transcriptomic landscape of the Stage 1 embryo. The 10X Genomics Visium™ spatial transcriptomics protocol identified nine clusters that mapped to seven embryonic regions: SAM tip, leaf and coleoptile; lower SAM and hypocotyl; suspensor; apical internal scutellum; basal internal scutellum; adaxial scutellum epidermis; and the abaxial scutellum epidermis. In the spatial transcriptomic UMAP, cell clusters derived from the shoot-root axis are separated from those of the scutellum, whereas cells from the scutellum epidermis are distinct from cells derived from the internal scutellum. Gene ontology (GO) analyses suggest that the scutellum epidermis functions in intercellular transport. In contrast, cells from the internal scutellum display carbohydrate and/or fatty acid metabolic functions. To overcome the lower cell-resolution issues of the current Visium technology (i.e., 1-10 cells), we integrated these spatial-transcriptomic data with scRNA-seq and LM-RNAseq analyses of Stage 1 embryos to generate a transcriptomic consensus. Consistent with the Visium UMAP, the scRNA-seq UMAP also shows separation of the epidermal and internal scutellum cell clusters. The combined transcriptomic consensus reveals that cells from the foliar leaf and coleoptile cluster together, and are transcriptomically distinct from the scutellum and the suspensor. Moreover, higher *wox3* mutations have similar phenotypic effects on the leaf and coleoptile, which supports the proposed homology of these maize embryonic organs.

Funding acknowledgement: National Science Foundation (NSF)

P53

The Auxin Response Factor ARF27 is required for maize root morphogenesis

(submitted by Linkan Dash <linkan@iastate.edu>)
Full Author List: Dash, Linkan[1]; McReynolds, Maxwell[2]; Draves, Melissa[1]; Khangura, Rajdeep[3 4]; Cowling, Craig[1]; Lang, Michelle[1]; Dilkes, Brian P.[3 4 5]; Walley, Justin W.[2]; Kelley, Dior R.[1]
[1] Department of Genetics, Development and Cell Biology, Iowa State University, Ames, Iowa 50011
[2] Department of Plant Pathology, Iowa State University, Ames, Iowa 50011
[3] Center for Plant Biology, Purdue University
[4] Department of Biochemistry, Purdue University
[5] Department of Horticulture and Landscape Architecture, Purdue University

Auxin response factors (ARFs) are a family of evolutionarily conserved transcription factors across the plant kingdom and are required for auxin signaling. Loss of *ARF* function in *Arabidopsis, Marchantia* and *Physcomitrium* has demonstrated that these transcription factors are required for many aspects of plant growth and development. In contrast, we do not know the roles of maize *ARFs* in plant morphogenesis. Here we describe a novel function for ZmARF27 in root hair development, primary root growth, lateral root formation, and auxin responses using reverse genetics and molecular approaches. Transcriptomic analysis of *arf27* indicates that this transcription factor is essential for integrating auxin-induced gene expression required for proper root development. In addition, ZmARF27 is a candidate gene for auxin mediated root growth in maize that we identified form a genome wide association study (GWAS) with >600 genotypes from the Wisconsin Diversity panel. Potential target genes of ZmARF27 have been reconstructed from a gene regulatory network (GRN) and validated with available DAP-seq data. A working model for the role of ZmARF27 as a key genetic driver of root morphogenesis provides a novel selection target for the genetic improvement of maize.

Gene / Gene Models described: *arf27*; Zm00001d045026
Funding acknowledgement: United States Department of Agriculture (USDA)

P54

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

(submitted by Hailong Yang <hailongyang@umass.edu>)
Full Author List: Yang, Hailong[1]; Gallagher, Joseph P[1]; De Neve, Amber[1]; Bartlett, Madelaine[1]
[1] Department of Biology, University of Massachusetts Amherst, Amherst MA, USA 01003

The evolution of cis-regulatory regions is critical for evolutionary divergence in gene function. 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 maize domestication gene involved in repressing growth in multiple developmental contexts, including in lateral buds and in 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 to determine how GT1's pleiotropic functions are regulated. These paths will help us to modulate gene expression in the grasses and understand the evolution of this important, pleiotropic domestication gene.

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

P55

The combination of morphogenic regulators BABY BOOM and GRF-GIF improves maize transformation efficiency

(submitted by Zongliang Chen <zlchen@waksman.rutgers.edu>)
Full Author List: Chen, Zongliang[1]; Debernardi, Juan M.[2 3]; Dubcovsky, Jorge[2 3]; Gallavotti, Andrea[1 4]
[1] Waksman Institute of Microbiology, Rutgers University, Piscataway, NJ.
[2] Department of Plant Sciences, University of California Davis, Davis, CA.
[3] Howard Hughes Medical Institute, Chevy Chase, MD.
[4] Department of Plant Biology, Rutgers University, New Brunswick, NJ

Transformation is an indispensable tool for plant genetics and functional genomic studies. While stable plant transformation is no longer a significant obstacle in maize, there is a continued need for accessible and efficient methods in academic laboratories. Here we present the GGB transformation system, a rapid and highly efficient transformation system optimized for the immature embryo transformation of two maize genetic backgrounds, including the inbred line B104. The combination of distinct morphogenic factors, the maize BABY BOOM transcriptional regulator (ZmBBM/EREB53) and the wheat GRF4-GIF1 (GROWTH REGULATING FACTOR4 – GRF-INTERACTING FACTOR1) chimera, together with a slightly modified QuickCorn protocol, regenerated transformed maize seedlings in approximately two months with an efficiency of 26 to 37%; notably, the efficiency was 10-fold higher than with using either component in isolation. Furthermore, the expression of both morphogenic factors in B104 did not significantly affect plant development, particularly on fertility, eliminating the need to remove the morphogenic regulators after *Agrobacterium* infections. We were also successful at quickly regenerating plantlets using seedling leaf segments of GGB-transformed B104 plants, while wild-type B104 plants failed to produce the same response, indicating that the presence of both regulators can enhance regeneration from vegetative tissue.To verify the efficiency of the GGB transformation system for genome editing, we designed a single guide RNA to target three maize *LITTLE ZIPPER* (*ZPR*) genes, *ZmZPR3*, *ZmZPR4a*, and *ZmZPR4b*. All the T0 generation plants with simultaneous disruptions in the three genes (triple-knockouts) had small leaf angles and appeared defective in ligule formation in both Hi-II and B104 backgrounds. Further genetic analysis indicated that *ZmZPR3* and *ZmZPR4a* are required to form the ligule and regulate leaf angle, while the role of *ZmZPR4b* is dispensable. Our research revealed a new role of *ZPR* genes in controlling ligule formation and leaf angle, an important architectural trait for high-density planting.

Funding acknowledgement: National Science Foundation (NSF)

P56

The enhancer of *spi1* (*eos1*) gene is involved in auxin regulated maize inflorescence development

(submitted by Prameela Awale <pa96f@mail.missouri.edu>)
Full Author List: Awale, Prameela[1]; Robil, Janlo[1]; Darnell, Zoe[1]; Hohenberger, Amy[1]; Lincoln, Calvin N.[1]; Coats, Diana[1]; McSteen, Paula[1]
[1] Division of Biological Sciences, University of Missouri-Columbia, MO

In maize, auxin is required for the initiation of all axillary meristems (branch, spikelet pair, spikelet, and floral meristem) in the tassel and the ear. Mutations in genes involved in auxin synthesis, transport or signaling can cause defects in all types of axillary meristems in the maize inflorescence. *enhancer of spi1* (*eos1*) mutants are defective in tassel branching and have a barren patch on the main spike, indicating that some branches are missing. However, no obvious phenotypic defects are seen in the rest of the tassel and ear. The auxin biosynthesis mutant *sparse inflorescence 1* (*spi1*) makes few tassel branches and defective spikelets in the tassel, whereas the ear is small and contains a barren patch. The auxin transport mutant *barren inflorescence 2* (*bif2*) make fewer branches than *spi1* and does not form an ear. *eos1;spi1* double mutants show a synergistic interaction with severe tassel phenotypes that includes reduced tassel length with few or no branches and empty spikelets, and a small ear with a large barren patch compared to *spi1*. In contrast, *eos1;bif2* double mutants look like *bif2*. These results suggest that *eos1* is involved in the auxin pathway that controls inflorescence architecture in maize.

Funding acknowledgement: National Science Foundation (NSF)

P57

The establishment of the anther somatic niche with single cell sequencing

(submitted by Blaine Marchant <dbmarchant@gmail.com>)
Full Author List: Marchant, D. Blaine[1]; Walbot, Virginia[1]
[1] Stanford University; Stanford, CA, USA 94305

The anther is the developmental housing of pollen and therefore the male gametes of flowering plants. The meiotic cells from which pollen are derived must differentiate *de novo* from somatic anther cells and synchronously develop with the rest of the anther. Anthropogenic control over anther development has become crucial for global agriculture so as to maintain inbred lines and generate hybrid seeds of many crops. Understanding the genes that underlie the proper differentiation, developmental landmarks, and functions of each anther cell type is thus fundamental to both basic and applied plant sciences. We investigated the development of the somatic niche of the maize (*Zea mays*) anther using single-cell RNA-seq (scRNA-seq). Extensive background knowledge on the birth then pace and pattern of cell division of the maize anther cell types and published examples of cell-type gene expression from *in situ* hybridization allowed us to identify the primary cell types within the anther lobe, as well as the connective cells between the four lobes. We established the developmental trajectories of somatic cell types from pre-meiosis to post-meiosis, identified putative marker gene for the somatic cell types that previously lacked any known specific functions, and addressed the possibility that tapetal cells sequentially redifferentiate. This comprehensive scRNA-seq dataset of the somatic niche of the maize anther will serve as a baseline for future analyses investigating male-sterile genotypes and the impact of environmental conditions on male fertility in flowering plants.

Funding acknowledgement: National Science Foundation (NSF)

P58

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

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

A critical transition in the life cycle of maize is the switch from vegetative growth, when the shoot apical meristem (SAM) produces leaves, to reproductive growth, when the SAM produces floral organs. This switch, called the floral transition, happens early in development and both internal cues and external signals, like photoperiod and temperature, influence the timing of when the SAM is reprogrammed. The timing of the floral transition is a major determinant of flowering– when the plant sheds pollen and exerts silks – which is an important adaptive trait and target of selection. Maize adapted to temperate latitudes has been selected to be photoperiod insensitive and will flower about the same time under short or long day environments but tropical maize retains much of the photoperiod sensitivity of its progenitor teosinte and requires short days to flower within the same time period as temperate maize. To better understand the photoperiod sensitivity of tropical maize and its impact on several developmental traits, specific temperate and tropical maize genotypes were characterized in field conditions in Hawaii (short days) and Iowa (long days). This poster will report our results on how growing environment influenced the timing of SAM reprogramming, total plant leaf number, and the number of days to flowering in these genotypes. We also analyzed the combination of photoperiod and temperature on the timing of flowering. Determining how these genotypes behave in different environments will provide the base knowledge that will guide our future analyses of the molecular mechanisms that differentiate photoperiod sensitive from photoperiod insensitive maize varieties and to identify which genes are important to regulate photoperiod sensitivity in tropical maize.
Keywords: maize, flowering time, photoperiod, floral transition, meristem

Funding acknowledgement: National Science Foundation (NSF)

P59

The redundancy paradox: uncovering the mechanisms of active compensation between paralogous genes in the maize meristem

(submitted by Sessen Daniel Iohannes <iohannes@cshl.edu>)
Full Author List: Iohannes, Sessen Daniel[1]; Jackson, David[1]
[1] Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 11724

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. One of the greatest paradoxes in evolutionary genomics is the retention of redundancy among ancient paralogous genes despite the accumulation of mutations. Genetic studies in yeast and plants have suggested that the ability of ancient paralogs to be redundant and to actively compensate for a loss of function does not depend on their ability to be highly co-regulated, but to be reprogrammed. My research work focuses on the maize trehalose-6-phosphate phosphatases RAMOSA3 (RA3) and TREHALOSE PHOSPHATE PHOSPHATASE 4 (TPP4), two important meristem development regulators, as a model for studying the reprogramming of paralogs. By using gene editing and chromatin accessibility assays, my work is investigating the hypothesis that non-coding sequences conserved across phylogenetic families over evolutionary time control active compensation by binding to factors that regulate gene expression. Furthermore, my work addresses whether the reprogramming of paralogs is linked to the stabilization of their mRNAs following the destabilization of a duplicate, therefore establishing a possible role for post-transcriptional regulation of compensation. Understanding the transcriptional and post-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.

Funding acknowledgement: National Science Foundation (NSF)

P60

Trans-activation analysis of maize anther transcription factors using protoplasts

(submitted by Lily O'Connor <loconnor@danforthcenter.org>)
Full Author List: O'Connor, Lily E.[1 2]; Zhan, Junpeng[2]; Marchant, D. Blaine[3]; Teng, Chong[2]; Walbot, Virginia[3]; Meyers, Blake C.[2 4]
[1] Department of Biology, Washington University, Saint Louis, MO, 63130, USA
[2] Donald Danforth Plant Science Center, Saint Louis, MO, 63132, USA
[3] Department of Biology, Stanford University, Stanford, CA, 94305, USA
[4] Division of Plant Science and Technology, University of Missouri, Columbia, MO, 65211, USA

Anthers are the male reproductive organ of flowering plants, producing the male gametophyte, pollen. Cell differentiation of maize anthers results in four concentric somatic cell layers surrounding central germinal cells. The development of each cell layer is important for proper pollen development, as many maize mutants with defects in somatic cell layers are male sterile. Over the course of the development of maize anthers, over 2000 transcription factor (TF) genes are expressed. Here, we use maize leaf protoplast trans-activation assays to identify targets of TFs involved in anther development. We show that a few of the anther TFs are sufficient to ectopically activate their endogenous targets either individually or in combination. We also propose a novel method for exploring TF combinations and gene regulatory networks using single cell RNA sequencing of protoplasts. This method could potentially be applied to study co-expressed TFs regulating many biological processes beyond anther development and from any plant species with defined protoplast transfection methods.

Funding acknowledgement: National Science Foundation (NSF)

P61 @amberdnv

Trehalose-6-phosphate-phosphatases have a conserved role shaping Panicoid inflorescence architecture

(submitted by Amber de Neve <adeneve@umass.edu>)
Full Author List: de Neve, Amber[1]; Yang, Jiani[2]; Higgins, Isabella[1]; Nuesslein, Benedikt[1]; Eveland, Andrea[2]; Bartlett, Madelaine[1]
[1] University of Massachusetts Amherst; Amherst, MA, USA 01003
[2] Donald Danforth Plant Science Center; Olivette, MO, USA 63132

Lateral axillary branching likely evolved in the Early Devonian ~400 million years ago, when the first seed and tree-like plants were starting to appear on Earth. In axillary branching, branches emerge from the axils of leaves. These branches can remain dormant as buds, or they can grow out to form shoots. Many mechanistic aspects of branch dormancy and growth are still mysterious, especially the role of Trehalose-6-Phosphate, which is present in low levels in all plants. *RA3*, which encodes a Trehalose-6-Phosphate Phosphatase, inhibits the growth of axillary branches in the maize inflorescence, allowing for the formation of short determinate spikelet pairs instead of long, indeterminate branches. To see if *RA3* function is conserved in grasses, we studied *brl2*, a *Setaria viridis* mutant with a compact, barrel-like inflorescence that maps to *SvRA3*. Through detailed phenotyping, including high-throughput computer vision techniques, we observed both similarities and differences in *ra3* phenotypes between maize and setaria. Our findings suggest that the function of *RA3* is conserved in *Setaria viridis* - that *SvRA3* also maintains the determinacy of inflorescence branches. Interestingly, *brl2* phenotypes suggest that *SvRA3* may also have a conserved role in keeping axillary branches dormant.

Gene / Gene Models described: *ra3, brl2*; Zm00001eb327910, Sevir.2G407500
Funding acknowledgement: National Institutes of Health (NIH), National Science Foundation (NSF), UMass Amherst Biology Graduate Program

P62

Untangling the effects of ethylene in maize vegetative shoots through single-cell transcriptomics

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

Phytohormones regulate numerous aspects of plant growth and development. Ethylene functions largely to inhibit growth and is a pivotal stress signal perceiving a diverse set of environmental conditions. Downstream of perception and signal transduction of this hormone, the *ETHYLENE INSENSITIVE3* (*EIN3/EIL*) genes are the first class of transcription factors to regulate the expression of ethylene response genes. We characterized independent mutations in *ZmEIL1* and *ZmEIL9* and found that plant growth is altered in the mutants, as well as development of leaves and internodes. We utilized single-cell transcriptomic analysis of vegetative shoot apices to uncover the impact of ethylene in maize seedlings. We generated individual datasets composed of *Zmeil* and normal shoots with and without the ethylene precursor ACC. Over 13,000 high-quality cells were obtained and grouped into 13 different clusters. We identified cell-specific genetic signatures in each cluster that separated within epidermal, indeterminate, primordia and vasculature lineages. Genes implicated in growth regulation, phytohormone signaling and stimuli response are mis-expressed in cell clusters between genotype and treatment conditions, suggesting key roles for *ZmEIL1;9* in coordinating the regulation of other developmental pathways in certain cell types. Through collaborative efforts, we leveraged DAP-seq to generate genome-wide *in vitro* ZmEIL:DNA binding maps. ZmEIL1 and ZmEIL9 display high degree of binding site overlap, suggesting many putative target genes are transcriptionally co-regulated, including a proportion of which are differentially expressed in our single-cell data. Collectively, this work provides novel insights into our understanding of the molecular response during ethylene signaling in maize.

Funding acknowledgement: National Science Foundation (NSF), NCSU Start-Up Funds

P63

bds1 and *bds2* redundantly regulate inflorescence and shoot architecture in maize via brassinosteroid biosynthesis

(submitted by Brian Zebosi <bzebosi@iastate.edu>)
Full Author List: Brian, Zebosi[1 2]; Erica, Unger-Wallace[1]; B. Best, Norman[3 4]; Wimalanathan, Kokulapalan[1 2]; Vollbrecht, Erik[1 2]
[1] Department of Genetics, Development and Cell Biology
[2] Interdepartmental Genetics and Genomics, Iowa State University
[3] 3Bond Life Sciences Center, University of Missouri, Columbia
[4] USDA-ARS, Columbia

Shoot architecture is a key determinant of grain yield in maize. Among the major plant growth regulators, brassinosteroids (BRs) affect multiple developmental processes and plant architecture traits, including organ size, sex determination, and leaf angle. However, genetic mechanisms by which BRs regulate these traits in maize remain poorly understood. We recently generated and characterized a recessive, EMS-induced maize mutant that we named *brassinosteroid deficient semi-dwarf mutant1* (*bds1*). *bds1-ref* mutants are semi-dwarf due to compressed internodes and are partially rescued by brassinolide. Mutants also have short leaf sheaths and twisted leaf blades and display a partial tassel-seed phenotype in the Mo17 background and reduced tassel branch number in B73. We localized bds1 to a small genomic region containing a nonsense mutation using map-based cloning and whole-genome sequencing. Non-complementation of *bds1-ref* and *bds1-Mu* alleles confirmed that *bds1* encodes a cytochrome p450 enzyme likely involved in brassinosteroid biosynthesis. Using phylogenetic and blast analysis, we identified that *bds1* has a close homolog. We named this gene *bds2* and generated several mutant alleles by remobilizing a nearby *Ds* transposable element. *bds2* single mutant plants appear normal, while *bds1-ref*;*bds2-Ds* double mutants are severely dwarfed with defects similar to those observed in BR-deficient mutants *nana1* and *nana2*. To understand the genetic interaction between brassinosteroids and jasmonic acid, we generated double mutants between *bds1-R* and *tasselseed2* (*ts2*), where we observed a synergistic interaction. The *bds1-R;ts2* double mutants have dramatically increased tassel feminization and reduced plant height and tassel branch number, suggesting that both BR and JA biosynthesis are required for sex determination and plant architecture regulation. Metabolite accumulation profiling analysis and feeding experiments are ongoing to confirm and characterize how the *bds1* and *bds2* mutants disrupt brassinosteroid biosynthesis. Based on these results, we propose that *bds1* and *bds2* cooperatively regulate shoot architecture and brassinosteroid biosynthesis.

Funding acknowledgement: National Science Foundation (NSF)

P64

lateral rootless2 (lrs2) functions in maize root development

(submitted by Colin Wilburn <Crw8hn@umsystem.edu>)
Full Author List: Wilburn, Colin R[1]; Awale, Prameela[1]; Robil, Janlo[1]; Coats, Diana[1]; McSteen, Paula[1]
[1] University of Missouri-Columbia; 1201 Rollins St; Columbia, MO, 65211

The growth hormone Auxin, or Indole Acetic Acid, plays a key role in the growth and development of plants . Auxin functions in phototropism, gravitropism, and root, leaf, and flower formation . Auxin is regulated through synthesis, transport, signaling and response. The *lrs2* and similar mutants have been found to alter auxin regulation. In preliminary data from our lab, the *lrs2* mutant exhibits a lack of lateral roots and seminal roots, and a substantially shorter primary root. Further investigation into the *lrs2* mutant phenotype and its relationship with the *bif2* mutant will provide a greater understanding of the maize auxin transport pathway.

Funding acknowledgement: National Science Foundation (NSF)

P65 @MaizeGDB

MaizeGDB: Maize protein structure resources

(submitted by Carson Andorf <carson.andorf@usda.gov>)
Full Author List: Andorf, Carson M[1 2]; Portwood, II, John L[1]; Sen, Shatabdi[3]; Hayford, Rita K[1]; Cannon, Ethalinda KS[1]; Gardiner, Jack M[4]; Woodhouse, Margaret R[1]
[1] USDA-ARS, Corn Insects and Crop Genetics Research Unit, Ames, IA 50011, USA
[2] Department of Computer Science, Iowa State University, Ames, IA 50011, USA
[3] Department of Plant Pathology & Microbiology, Iowa State University, Ames, IA 50011, USA
[4] Division of Animal Sciences, University of Missouri, Columbia, MO 65211, USA

MaizeGDB offers new tools to accelerate protein structural comparisons between maize and other plants as well as human and yeast outgroups. These tools leverage recent technological breakthroughs in protein structure prediction, such as the release of AlphaFold and ESMFold, which have reduced the structural biology bottleneck by several orders of magnitude. MaizeGDB also offers bulk downloads of comparative protein structure data, along with predicted functional annotation information, to assist maize researchers in assessing functional homology, gene model annotation quality, and other information unavailable to maize scientists even a few years ago. A new method called Functional Annotations using Sequence and Structure Orthology (FASSO) combines sequence- and structure-based approaches to obtain a more accurate and complete set of orthologs across diverse species, and was used to annotate orthologs between five plant species (maize, sorghum, rice, soybean, Arabidopsis) and three distance outgroups (human, budding yeast, and fission yeast) resulting in over 270,000 functional annotations across the eight proteomes, including annotations for over 5,600 uncharacterized proteins. FASSO also provides confidence labels on ortholog predictions and flags potential misannotations in existing proteomes and demonstrates the utility of the approach by exploring the annotation of the maize proteome.

Funding acknowledgement: United States Department of Agriculture (USDA)

P66

Pan-genome data at MaizeGDB

(submitted by Ethalinda Cannon <Ethy.Cannon@usda.gov>)
Full Author List: Cannon, Ethalinda KS[1]; Woodhouse, Margaret R[1]; Portwood II, John L[1]; Gardiner, Jack M[2]; Hayford, Rita K[1]; Andorf, Carson M[1]
[1] USDA-ARS, Corn Insects and Crop Genetics Research Unit, Ames, IA 50011, USA
[2] University of Missouri, Division of Animal Sciences, CAFNR, Columbia, MO 65211, USA

With increasing numbers of reference-quality genomes, especially the valuable set of the 25 NAM founders and B73 reference assemblies, pan-genome data, analyses, and research within Zea mays becomes increasingly accessible, permitting expanded research across maize diversity. The Pan-Androgonaceae sequencing and annotation project will add to efforts to create pan-grass genomes and analyses, enabling comparative research across multiple grass species. MaizeGDB is adopting and developing new software and methods for generating, accessing, and exploring pan-genome data. This poster will describe the new pan-genome section at the MaizeGDB website, pan-genome improvements to the genome browsers, and a powerful exploration tool, the Genome Context Viewer (GCV).

Funding acknowledgement: United States Department of Agriculture (USDA)

P67

MaizeMine: New tools for *Zea mays* pangene data mining

(submitted by Jack Gardiner <jack.m.gardiner@gmail.com>)
Full Author List: Gardiner, Jack[1]; Triant, Deborah[1]; Walsh, Amy[1]; Andorf, Carson[2]; Elsik, Christine[1 3]
[1] Division of Animal Sciences, University of Missouri, Columbia MO 65211, USA
[2] USDA-ARS Corn Insects and Crop Genetics Research Unit, Iowa State University, Ames, IA 50011, USA
[3] Division of Plant Sciences, University of Missouri, Columbia MO 65211, USA

Increasingly, there is a need for maize researchers to conduct comparative analysis between their own datasets and published or publicly available data. MaizeGDB's data mining warehouse, MaizeMine (http://maizemine.maizegdb.org), utilizes the InterMine data warehousing platform which empowers researchers without bioinformatics skills to integrate their data with publicly available data and perform meta-analyses using a suite of tools, including a keyword search, built-in template queries with intuitive search menus, and a QueryBuilder tool for creating custom queries. The List tool allows users to upload identifiers to perform set operations and to execute template queries with lists. Users can easily compare their results with published results by uploading genomic coordinates or identifiers. Here we report the updating of MaizeMine (v1.5) with the addition of genome assemblies of twenty-five *Zea mays* NAM founder lines, along with a pangene dataset encompassing all the lines. Search tools enable users to query for syntelogs across *Z. mays* lines, and to identify genes unique to lines. In addition, all genomes are annotated with gene symbols, descriptions, Gene Ontology annotations and pathways. MaizeMine also continues to integrate the *Z. mays* B73 genome with GO and protein annotations from UniProt, protein domains from InterPro, homologs and SNP from Ensembl Plants, pathways from KEGG and Plant Reactome, gene expression from MaizeGDB's qTeller, and project-specific data generated by the maize research community. Gene alias identifiers facilitate easy conversion between the old and new B73 gene sets, and cross references are provided to link the B73 Zm00001eb.1 gene set with RefSeq.

Funding acknowledgement: United States Department of Agriculture (USDA)

P68

Stress response functional annotation using RNA expression in maize

(submitted by Rita Hayford <ritakusiappiah@gmail.com>)
Full Author List: Hayford, Rita K[1]; Woodhouse, Margaret R[1]; Portwood, John L[1]; Sen, Shatabdi[2]; Gardiner, Jack M[3]; Cannon, Ethalinda K[1]; Andorf, Carson M[1 4]
[1] USDA-ARS, Corn Insects and Crop Genetics Research Unit, Ames, IA 50011, USA
[2] Department of Plant Pathology & Microbiology, Iowa State University, Ames, IA 50011, USA
[3] Division of Animal Sciences, University of Missouri, Columbia, MO 65211, USA
[4] Department of Computer Science, Iowa State University, Ames, IA 50011, USA

Maize (Zea mays ssp. mays) is a major crop widely grown throughout the world. In addition to food, maize is used as fuel and as feed for animals. In spite of the diverse use of maize, this important crop is exposed to several environmental cues reducing yield and quality. Transcriptome profiling studies have been used to provide insights into the molecular mechanisms underlying stress response. Although MaizeGDB browsers and tools have been used to assist with the functional annotation of genes, there still remain unknown functions of many genes. To enhance the functional annotation of maize-specific genes, MaizeGDB has outlined a data-driven approach with emphasis on identifying genes and traits related to biotic and abiotic stress. Many RNA-Seq reads have been mapped to older versions of the reference genomes which could lead to omission of gene model annotations. Hence, our goal is to map high quality RNA-Seq expression reads to the recent version of the reference genome B73 (B73v5) and to deduce stress-related functional annotation of gene models. Publicly available RNA-Seq datasets related to biotic and abiotic stress generated from seeds of B73 cultivar were used in this analysis. We use heat stress as a case study to illustrate the use of the pipeline and its implications. In the future, we will conduct meta-analysis of all mapped datasets to identify common differentially expressed genes and pathways from both biotic and abiotic data. Our analysis will facilitate identifying multiple stress response gene models and annotation in maize.

Funding acknowledgement: United States Department of Agriculture (USDA)

P69 @ShatabdiSen2

Maize Feature Store (MFS): A centralized resource to manage and analyze curated maize multi-omics features for machine learning applications.

(submitted by Shatabdi Sen <shatabdi@iastate.edu>)
Full Author List: Sen, Shatabdi[1]; Woodhouse, Margaret R[2]; Portwood, John L[2]; Andorf, Carson M[2 3]
[1] Department of Plant Pathology & Microbiology, Iowa State University, Ames, IA 50011, USA
[2] USDA-ARS, Corn Insects and Crop Genetics Research Unit, Ames, IA 50011, USA
[3] Department of Computer Science, Iowa State University, Ames, IA 50011, USA

The big-data analysis of complex data associated with maize genomes accelerates genetic research and improves agronomic traits. As a result, efforts have increased to integrate diverse datasets and extract meaning from these measurements. Machine learning models are a powerful tool for gaining knowledge from large and complex datasets. However, these models must be trained on high-quality features to succeed. Currently, there are no solutions to host maize multi-omics datasets with end-to-end solutions for evaluating and linking features to target gene annotations. Our work presents the Maize Feature Store (MFS), a versatile application that combines features built on complex data to facilitate exploration, modeling, and analysis. Feature stores allow researchers to rapidly deploy machine learning applications by managing and providing access to frequently used features. We populated the MFS for the maize reference genome with over 14,000 gene-based features based on published genomic, transcriptomic, epigenomic, variomic, and proteomics data sets. Using the MFS, we created an accurate pan-genome classification model with an AUC-ROC score of 0.85. The MFS is publicly available through the maize genetics and genomics database.
Database URL: https://mfs.maizegdb.org/

Funding acknowledgement: United States Department of Agriculture (USDA)

P70 @SchnableLab

A common resequencing-based genetic marker dataset for global maize diversity

(submitted by Jonathan Turkus <jturkus2@unl.edu>)
Full Author List: Grzybowski, Marcin W[1 2 3]; Mural, Ravi V[1 2]; Xu, Gen[1 2]; Turkus, Jonathan D[1 2]; Yang, Jinliang[1 2]; Schnable, James C[1 2]
[1] Center for Plant Science Innovation - University of Nebraska-Lincoln; Lincoln, NE, USA, 68503
[2] Department of Agronomy and Horticulture - University of Nebraska-Lincoln; Lincoln, NE, USA, 68503
[3] Department of Plant Molecular Ecophysiology - Institute of Plant Experimental Biology and Biotechnology - Faculty of Biology - University of Warsaw, Warsaw, Poland

Maize (*Zea mays ssp. mays*) populations exhibit vast amounts of genetic and phenotypic diversity. As sequencing costs have declined, an increasing number of projects have sought to measure genetic differences between and within maize populations using whole genome resequencing strategies, identifying millions of segregating single-nucleotide polymorphisms (SNPs) and insertions/deletions (InDels). Unlike older genotyping strategies like microarrays and genotyping by sequencing, resequencing should, in principle, frequently identify and score common genetic variants. However, in practice, different projects frequently employ different analytical pipelines, often employ different reference genome assemblies, and consistently filter for minor allele frequency within the study population. This constrains the potential to reuse and remix data on genetic diversity generated from different projects to address new biological questions in new ways. Here we employ resequencing data from 1,276 previously published maize samples and 239 newly resequenced maize samples to generate a single unified marker set of ~366 million segregating variants and ~46 million high confidence variants scored across crop wild relatives, landraces as well as tropical and temperate lines from different breeding eras. We demonstrate that the new variant set provides increased power to identify known causal flowering time genes using previously published trait datasets, as well as the potential to track changes in the frequency of functionally distinct alleles across the global distribution of modern maize.

Funding acknowledgement: National Science Foundation (NSF), United States Department of Agriculture (USDA), Department of Energy (DOE), National Science Center (Poland), Nebraska Research Initiative

P71 @mizeroolivier99

A deep learning object detection pipeline enables rapid detection of maize kernels

(submitted by Olivier Niyonshuti Mizero <niyomizer@huskers.unl.edu>)
Full Author List: Niyonshuti Mizero, Olivier[1 2]; Christenson, Clay[1 2]; Tross, Michael[1 2]; Schnable, James C.[1 2]; Sigmon, Brandi[3]
[1] Department of Agronomy and Horticulture; University of Nebraska-Lincoln; Lincoln; Nebraska; USA; 68583
[2] Plant Science Innovation; University of Nebraska-Lincoln; Lincoln; Nebraska; USA; 68588
[3] Department of Plant Pathology; University of Nebraska-Lincoln; Lincoln; Nebraska; USA; 68583

Grain yield for maize depends largely on the size, and number of kernels on ears. The number of kernels per ear is in turn determined by the number of kernel rows per ear and the number of kernels in each row. By measuring these yield component traits, their genetic bases can be studied and improved. However, existing methods for phenotyping kernel traits are often labor intensive and/or have limited accuracy. Here, we describe a low-cost and straightforward phenotyping system consisting of a maize ear scanner and an image processing pipeline that can be used to predict, count, and measure kernel features on diverse maize ears. The maize ear scanner was used to obtain videos of rotating maize ears. These videos were then flattened to provide 2D digital images of the entire surface of the ears. These digital images were processed to generate kernel-specific traits using a deep-learning based computer vision pipeline developed in TensorFlow**.** Transfer learning was used to develop a deep learning object detection model with a CNN architecture, specifically Mask-RCNN, with Inception Resnet50 to balance accuracy and speed for the overall performance on our dataset. The training dataset consisted of a subset of 122 digital images with 124,405 kernel instances from ears collected among two maize diversity panels grown in Nebraska during 2019 and 2020. A separate dataset of maize ear images annotated for kernel positions will be used to test model accuracy through the calculation of the mean average precision. This high-throughput and automated phenotyping pipeline will enable faster and more accurate prediction of kernels and kernel features on maize ears and may ultimately improve our understanding of genetics of maize yield component traits.

Funding acknowledgement: National Science Foundation (NSF), Nebraska Corn Board, Foundation for Food and Agricultural Research

P72

A time-series analysis of Azimuthal Canopy Orientation Architecture using UAV images

(submitted by Jesse Hickman <jhickman@iastate.edu>)
Full Author List: Zhou, Yan[1]; Hickman, Jesse[2]; Schnable, Patrick[1]
[1] Department of Agronomy, Iowa State University, Ames, Iowa, 50011
[2] Department of Biochemistry, Biophysics, and Molecular Biology - AGLS, Ames, Iowa, 50011

Canopy architecture is a major contributor to efficient interception of solar radiation. Several studies suggest some maize genotypes exhibit the ability to re-adjust their azimuthal canopy orientations in response to increasing planting density. Previous literatures measured leaf azimuthal orientation manually in the field which is time-consuming and challenging to collect at scale. Here we report a high throughput data collection approach using UAV-acquired images of azimuthal canopy orientations. Canopy data were collected at three developmental stages (40, 50 and 60 days after planting) and multiple planting densities. Data were collected from wild-type controls and mutants in genes shown via GWAS to be associated with azimuthal re-orientation. Azimuthal canopy orientations in the two wildtype controls exhibited increased frequency to re-orientate towards open interrow spaces as planting density increased. In contrast, such responses were reduced or even reversed in the mutants. Our results suggest that the ability of maize to readjust canopy orientations is potentially regulated by genes participating in light and auxin signaling pathways.

P73 @AnnaHaber8

An improved genome assembly and annotation of the tetraploid resurrection grass Eragrostis nindensis

(submitted by Anna Pardo <haberan2@msu.edu>)
Full Author List: Pardo, Anna CH[1 2]; Pardo, Jeremy D[1 3]; VanBuren, Robert[1 2]
[1] Department of Horticulture, Michigan State University, East Lansing, MI, USA 48824
[2] Plant Resilience Institute, Michigan State University, East Lansing, MI, USA 48824
[3] Department of Plant Biology, Michigan State University, East Lansing, MI, USA 48824

Drought is a major constraint on agriculture and a historical driver of plant adaptation and diversification. Arguably, the most extreme adaptation to water deficit can be found in the desiccation-tolerant, so-called “resurrection” plant species. Eragrostis nindensis, a grass native to southern Africa, is the only desiccation-tolerant plant with a close crop relative, the Ethiopian and Eritrean staple cereal teff (Eragrostis tef). Insights from the comparative study of the molecular mechanisms of water deficit response in E. nindensis compared to desiccation sensitive species such as teff may be applicable to improving the climate resilience of teff and other cereals. High-quality genomic resources will be essential for the robust examination of desiccation tolerance mechanisms and identification of candidate genes for crop improvement. We produced a highly contiguous genome assembly of E. nindensis using PacBio HiFi long-read sequencing, and used Oxford Nanopore long-read RNA-sequencing data as evidence to improve the genome annotation. Using these improved resources, we have found that E. nindensis is a recent autotetraploid, the first confirmation of autopolyploidy in the resurrection plants to our knowledge. We are also using this genome in a study of the expression dynamics of late embryogenesis abundant (LEA) genes, which are key players in desiccation tolerance, in E. nindensis and other resurrection plants compared to desiccation sensitive species such as teff. This new version of the E. nindensis genome will be a useful tool for the community of desiccation tolerance researchers.

Funding acknowledgement: National Science Foundation (NSF)

P74

An improved maize Practical Haplotype Graph (PHG) to study and store maize diversity

(submitted by Cinta Romay <mcr72@cornell.edu>)
Full Author List: Romay, M Cinta[1]; Berthel, Ana H[1]; Bradbury, Peter J[2]; Gage, Joe L[3]; Johnson, Lynn C[1]; Miller, Zack R[1]; Valdes-Franco, J Arcadio[4]; Buckler, Ed S[1 2 4]
[1] Institute for Genomic Diversity, Cornell University, Ithaca, NY, USA 14853
[2] United States Department of Agriculture-Agricultural Research Service, Ithaca, NY, USA 14853
[3] Department of Crop and Soil Sciences, North Carolina State University, Raleigh, NC, USA 27695
[4] Plant Breeding and Genetics Section, School of Integrative Plant Science, Cornell University, Ithaca, NY, USA 14853,

Pangenomes provide novel insights for population and quantitative genetics, genomics, and breeding that are not available from studying a single reference genome. Unfortunately, managing and using pangenomes for genomically diverse species like maize is computationally and practically challenging. The Practical Haplotype Graph (PHG) is a trellis graph representation, anchored to the reference genome, developed to deal with this challenge. The PHG can handle large structural variation, efficiently store diversity, and impute complete genomes from low density sequence or variant data. The PHG encapsulates a database for storing sequence and alignment information, software for building and using that database, and pipelines that employ widely used third-party software for some of those tasks. To build a maize PHG, 86 genome assemblies were aligned using anchorwave to the B73 v5 assembly. The reference genome was divided into discrete regions based on the B73 v5 gff3 annotations, with end points selected in highly conserved regions where at least 23 of the NAM genomes have 10 or more conserved base pairs. Each assembly segment aligned to a reference region constitutes a haplotype. Haplotypes in genic regions with 0.0001 or less divergence and intergenic regions with 0.001 or less divergence were collapsed, replacing them with the consensus haplotype. Sequence reads from different germplasm including NAM, inbreds from the US national seed bank, and the 2014-2023 Genomes to Fields (G2F) materials, covering over 10 years of different technologies, were then aligned against the pangenome database to identify haplotypes matching each read. The imputed path through the graph was used to identify variants for each entry. Our results show that the maize PHG is a useful tool for research and breeding, achieving consistent and highly accurate genome imputation across materials and technologies, while storing the data in an accessible and space efficient way.

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

P75

Building a practical haplotype graph based genetic map of the zea synthetic population

(submitted by Tim Kosfeld <TKosfeld@danforthcenter.org>)
Full Author List: Kosfeld, Tim[1]; Ziegler, Greg[1]; Stetter, Markus[2]; Ross-Ibarra, Jeffrey[3]; Flint-Garcia, Sherry[4]; Baxter, Ivan[1]
[1] Donald Danforth Plant Science Center, St. Louis, MO 63132
[2] Institute for Plant Sciences and Center of Excellence on Plant Sciences, University of Cologne, Cologne, Germany
[3] Department of Evolution and Ecology, Center for Population Biology, and Genome Center, University of California, Davis, CA
[4] United States Department of Agriculture—Agricultural Research Service, Columbia, MO Division of Plant Sciences, University of Missouri, Columbia, MO

Domestication and inbreeding of modern maize has resulted in the loss of much 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 a high-resolution genetic resource for the study of teosinte has made it difficult to associate that variation with agronomic traits. We are producing a genetic map of the Zea Synthetic maize population, a genetic resource created by randomly mating a 38-accession synthetic breeding population consisting of 11 geographically diverse teosinte (Zea mays ssp. parviglumis) crossed with B73 and the 26 inbred Nested Association Mapping (NAM) founders. Our approach leverages low coverage GBS data and 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 the 26 inbred NAM founders and 11 teosinte pseudo-genomes. ~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 resolution GBS sequences from 2000 doubled haploids from the Zea Synthetic population to accurately impute WGS data for the entire population for use in association mapping.

P76

Characterization of the gene networks underlying cuticle production in maize silks via systems biology approaches

(submitted by Keting Chen <kchen@iastate.edu>)
Full Author List: Chen, Keting[1 2]; McNinch, Colton[3 4]; Guo, Yuxiang[1 2]; Dennison, Tes[4 5]; Mahgoub, Umnia[2]; Stefani, Skylar[2]; Dorman, Karin S.[1 2 7]; Nikolau, Basil J.[2 3 5 6]; Lauter, Nick[4 8]; Yandeau-Nelson, Marna D.[1 2 3 5]
[1] Department of Genetics, Development & Cell Biology, Iowa State University, Ames, Iowa
[2] Bioinformatics and Computational Biology Graduate Program, Iowa State University, Ames, Iowa
[3] Molecular, Cellular, and Developmental Biology Graduate Program, Iowa State University, Ames, Iowa
[4] Department of Plant Pathology & Microbiology, Iowa State University, Ames, Iowa
[5] Interdepartmental Genetics and Genomics Graduate Program, Iowa State University, Ames, Iowa
[6] Roy J. Carver Department of Biochemistry, Biophysics & Molecular Biology, Iowa State University, Ames, Iowa
[7] Department of Statistics, Iowa State University, Ames, Iowa
[8] Corn Insects and Crop Genetics Research Unit, USDA-ARS, Ames, Iowa

The plant cuticle is comprised of a cutin polymer matrix infused with and coated by cuticular waxes that together form a protective layer against environmental stresses. The cuticular waxes on maize silks are comprised of very-long-chain fatty acids (VLCFAs), and VLCFA-derivatives including aldehydes and hydrocarbons. These metabolites are linked via enzymatic reactions as presumed precursors, intermediates, and end-products for silk cuticular wax biosynthesis. Herein, we collected the silks from inbreds B73 and Mo17, and queried the cuticular wax metabolomes and transcriptomes along a spatio-temporal gradient that captures the developmental progression and the environmental transition as silks emerge from the husks. We noted that cuticular wax biosynthesis is sensitive to the transition of silk micro-environment and the genetic background, reflected by varying wax compositions between the husk-encased and emerged silks, and between inbreds B73 and Mo17. Joint statistical analysis of cuticular wax metabolomes and companion transcriptomes was then employed to dissect the gene network underlying the observed cuticular wax variations, and identified ~300 genes associated with the cuticular wax variation between B73 and Mo17, and along the silk length. These cuticular wax-associated genes include those confirmed to participate in cuticular wax biosynthesis and the ones from pathways that directly or indirectly interact with cuticular wax biosynthesis, including cell wall biogenesis, proteasome-mediated protein degradation, and vesicle trafficking. In addition, 42 IBMRILs that demonstrate broad variation in silk cuticular wax compositions were selected for expression-QTL mapping. The genome-wide eQTL distribution and the eQTL distribution for the cuticular wax-associated genes were thus identified, allowing for further exploration into the potential regulatory relationships within the gene networks identified by the multi-omics integration pipeline. In conclusion, this systems' biology approach identifies the gene network underlying silk cuticular waxes, demonstrating the complexity of metabolic context that can determine cuticle deposition.

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

P77

Cloudifying reproducible breeding pipelines: integrating TASSEL and the PHG with R and JupyterHub

(submitted by Brandon Monier <bm646@cornell.edu>)
Full Author List: Monier, Brandon[1]; Econopouly, Bethany F.[1]; Casstevens, Terry M.[1]; Agosto-Pérez, Francisco J.[2]; Bradbury, Peter J.[1 3]; Robbins, Kelly R.[2]; Buckler, Edward S.[1 2 3]
[1] Institute for Genomic Diversity, Cornell University, Ithaca, NY 14853
[2] Section of Plant Breeding and Genetics, Cornell University, Ithaca, NY 14853
[3] United States Department of Agriculture-Agricultural Research Service, Robert W. Holley Center for Agriculture and Health, Ithaca, NY 14853

Analyzing, translating, and applying genomic and genetic data is crucial for advancing crop improvement to achieve the nutritional and economic goals of the Global Food Security Strategy through USAID. Despite its significance towards global agriculture, the widescale integration of this type of data is limited for end-users at research institutions across African, Caribbean, and Latin American countries. One bottleneck many research institutions face is data processing capacities, which we can overcome using server-based infrastructure, applications, and new methodologies to reduce dimensionality for genomic predictors. Here, we present new methods for rTASSEL and rPHG: R interfaces to Trait Analysis by aSSociation, Evolution, and Linkage version 6 (TASSEL 6) and the Practical Haplotype Graph (PHG), respectively. These updates provide ways to connect users to breeding data management systems, query haplotype data, and perform genic allele activity analyses. Additionally, this architecture allows us to integrate these interfaces with JupyterHub, a platform for deploying, running, and sharing computational notebooks amongst research groups using server-based computational resources. Combining these resources will provide plant breeding and genetics groups with a shared platform for better reproducibility of informatics pipelines, enhanced computation, and a foundation for training, empowering, and teaching early-career scientists.

Funding acknowledgement: National Science Foundation (NSF), United States Department of Agriculture (USDA), Bill & Melinda Gates Foundation, United States Agency for International Development (USAID)

P78

Combining GWAS of metabolomic and transcriptomic datasets to study the impact of drought on plant growth and metabolism in *Sorghum* and *Setaria*

(submitted by Allen Hubbard <ahubbard@danforthcenter.org>)
Full Author List: Hubbard, Allen[1]; Connelly, Louis[1]; Li, Xiaoping[1]; Jiang, Hui[1]; Barrett, Jennifer[1]; Luebbert, Collin[1]; Pope, Maddison[1]; Khambampati, Shrikaar[1]; Baxter, Ivan[1]
[1] Donald Danforth Plant Science Center, St. Louis, Missouri, 63132

Plants make an amazing array of metabolites to grow and respond to environmental change. The large number of compounds created by plants are poorly characterized and the genetic programs controlling them are largely unknown. In order to better understand the metabolomic response of C4 plants to drought stress, we conducted parallel experiments in *Sorghum* and *Setaria* using diversity panels. Plants were grown in a controlled environment phenotyping system at two watering levels and samples were harvested 6 days after the watering levels were set. Metabolites for each sample were quantified in an untargeted fashion via LC-MS using two different columns in both positive and negative mode to identify a large number of compound classes. A third of the samples were also profiled for RNA transcripts. ~3800 metabolomics samples, each run on two columns in two modes created an immense informatics challenge. To improve the sensitivity and accuracy of metabolite detection in large datasets like ours, we have developed a suite of three computational tools to overcome the challenges of unreliable algorithms and inefficient validation protocols: isolock, autoCredential and anovAlign. isolock uses metabolite-istopolog pairs (isopairs) to calculate and correct for mass drift noise across LC-MS runs. autoCredential leverages statistical features of LC-MS data to amplify naturally present ^{13}C isotopologs and validate metabolites through isopairs. anovAlign, an anova-derived algorithm, is used to align retention time windows across samples to improve delineation of retention time windows for mass features. Using the I.A.A suite, we have quantified thousands of mass-features across the 3,800 metabolomics samples. Genome wide association analysis has identified a large number of loci affecting these metabolites, including several loci in syntenic regions of the *Setaria* and *Sorghum* genomes for the same metabolite. We are combining the loci with transcriptomic and genomic data to identify candidate genes and alleles underlying the metabolomic response to water deficit, as well as leveraging tandem mass spectrometry (Ms2) to better characterize promising mass features.
Funding acknowledgement: Department of Energy (DOE)

P79

Comparative genomic analysis of DNA replication in maize and sorghum

(submitted by Emily Wheeler <eamarkha@ncsu.edu>)
Full Author List: Wheeler, Emily[1]; Wear, Emily[1]; Mickelson-Young, Leigh[1]; Thompson, William[1]; Hanley-Bowdoin, Linda[1]
[1] Department of Plant and Microbial Biology, North Carolina State University, Raleigh, NC 27595

Nuclear DNA replication is a highly regulated process in which the time of replication of different loci integrates many aspects of genome structure and function, including transcriptional activity, chromatin structure, epigenetic state, and 3-D structure. Understanding this process is important because chromosomal events that occur early in development, and are coupled to DNA replication, may impact gene expression programs and observable traits throughout the plant life cycle. In mammalian systems replication timing is highly conserved across cell types and related species and in this project we examine the conservation of replication timing between maize and sorghum, specifically looking at syntenic regions and their respective chromosomal location. We also explore the relationship between replication timing and RNA transcription. This project defines regions in which replication is altered between maize and sorghum and starts the process of understanding how replication programs are genetically determined. The project will further establish replication time as an important integrative genomic annotation, and shed light on the functional compartmentation of plant genomes.

Funding acknowledgement: National Science Foundation (NSF)

P80

Comparison of methods for detection and quantification of tar spot foliar infection in maize using dynamic colorspace thresholding, object detection, and contour analysis

(submitted by Jordan Manchego <manchego@msu.edu>)
Full Author List: Manchego, Jordan[1]; Colbry, Dirk[2]; Thompson, Addie[1]
[1] Department of Plant, Soil & Microbial Sciences; Michigan State University; East Lansing, MI 48824
[2] Department of Computational Math, Science, and Engineering; Michigan State University; East Lansing, MI 48824

The expanding geographic range of *Phyllachora maydis*, the fungus that induces Tar Spot infection on corn foliage, is increasingly threatening a Michigan industry that contributes over $1 billion to the state's economy annually. Foliar infection of maize by *P. maydis* is often difficult to detect early. Visible lesions initially appear tiny, ambiguous, and sparse, making them difficult to identify with the naked eye. Both farmers and breeders of corn would benefit from better tools that allow early, definitive detection of lesions and provide more time for management decisions. This tool must verify presence of *P. maydis* and quantify infection severity as quickly as possible to allow growers the most options for treatment. Advances in machine learning now enable quantification of crop infection presence and severity using powerful object detection packages. With the growing availability of open-source tools, such as the Mask Region-Based Convolutional Neural Network (Mask R-CNN) and PlantCV, the field of plant disease phenotyping has more options for methods than ever before. I propose comparing the accuracy of two potential pipelines to quantify tar spot infection severity: one based on heuristic methods, involving techniques such as dynamic image colorspace thresholding, and the other based on the use of annotations, such as object detection and contour analysis. Comparison of these two methods will provide insight into challenges involved with phenotyping in the field as well as phenotyping foliar diseases using automated methods.

Funding acknowledgement: National Science Foundation (NSF), United States Department of Agriculture (USDA), IMPACTS NRT, MSU Plant Science Fellowship

P81

DNA replication timing: A comparison of two genomic methods in maize root tips

(submitted by Leigh Mickelson-Young <lamickel@ncsu.edu>)
Full Author List: Mickelson-Young, Leigh[1]; Wear, Emily[1]; Song, Jawon[2]; Burroughs, Mason[1]; Thompson, William F.[1]; Hanley-Bowdoin, Linda[1]
[1] North Carolina State University, Department of Plant and Microbial Biology, Raleigh, NC 27595
[2] University of Texas at Austin, Texas Advanced Computing Center, Austin, TX 78758

DNA replication follows a temporal program in which different loci replicate at different times during S phase. Replication time is considered to be a functional read out of genomic features and chromatin organization. We previously used a protocol called "Repli-seq" to determine replication timing in maize B73 root tips (Mickelson-Young et. al. 2022). In Repli-seq, the thymidine analog, EdU, is incorporated into replicating DNA, and flow sorting is used to separate nuclei containing labeled DNA into early, middle, and late replicating fractions. Sequence data from immunoprecipitated EdU-labeled DNA in each of these fractions is then used to assess replication time for loci across the genome. In contrast, replication timing procedures based on DNA copy number use the relative copy number of a given locus in DNA from a population of asynchronously replicating nuclei to indicate its replication time, with earlier replicating sequences having higher copy numbers. Conventional DNA copy number procedures use unlabeled nuclei to compare a flow-sorted mid-S sample to nonreplicating G1 reference (S/G1 ratio). We modified the S/G1 technique to use nuclei from root tips pulse-labeled with EdU. This EdU-S/G1 procedure allows a much cleaner separation between replicating and non-replicating nuclei and a more complete sample of S phase. Relative to Repli-Seq, EdU-S/G1 uses less plant material, has shorter flow sorting times, and does not require immunoprecipitation of EdU-labeled DNA. Here we show that results from EdU-S/G1 are comparable to those of Repli-Seq, allowing us to quickly generate replication timing profiles in Sorghum and other maize cultivars such as NC350 for comparison to B73.

Funding acknowledgement: National Science Foundation (NSF)

P82

Deciphering the gene regulatory network underlying compensatory mechanisms of autophagy in maize

(submitted by Divya Mishra <dmishra5@wisc.edu>)
Full Author List: Marathe*, Sarika[1]; Mishra*, Divya[1]; McCalla, Sunnie Grace[1]; Shin, Junha[1]; Viestra, Richard[1]; Otegui, Marisa[1]; Roy, Sushmita[1]
[1] Department of Botany and Center for Quantitative Cell Imaging, University of Wisconsin-Madison

*The first two authors are listed as co-first authors.Autophagy is an essential cellular that replenishes nutrients by degrading unwanted proteins and organelles coordinately. One crucial step of autophagy is the lipidation of the gene ATG8, which is enhanced in association with another important gene ATG12, and promotes cargo capture, vesicle expansion, and closure. We generated atg12 mutants to understand the compensatory mechanisms that get activated without autophagy machinery. The atg12 mutants showed reduced autophagic transport and poor growth in a nutrient-deficient medium. Further, we measured atg12 mutant gene expression under various nutrient-deficient conditions, such as carbon and nitrogen starvation, and across various leaf developmental stages. We applied the gene regulatory network (GRN) inference algorithm MERLIN to our expression dataset to identify the regulatory programs activated under nutrient-rich and nutrient-poor conditions. We inferred a genome-scale GRN with 911 regulators connecting 7,280 genes and 7,008 predicted regulatory edges using MERLIN. Many MERLIN modules were enriched for relevant biological processes indicative of stress response and were differentially regulated between genotypes and conditions. Of particular note, four gene modules showed significant differential patterns that were conserved across multiple treatments. These modules were associated with the anthocyanin pathway, unfolded protein response, and biosynthetic pathways. Taken together, our network-based analysis identified condition-specific and common gene expression modules in atg12 mutants that can provide insight into the compensatory processes in autophagy-deficient condition

Funding acknowledgement: National Science Foundation (NSF)

P83 @BioBDub

Field and transcriptomic approach to understand hybrid specific response to N supplementation

(submitted by Brandon Webster <webst250@msu.edu>)
Full Author List: Webster, Brandon[1]; Ying, Sheng[2]; Gomez-Cano, Lina[2]; Newton, Linsey[1]; Cruz Gomez, Leidy Mariel[2]; Lundquist, Peter[2]; Grotewold, Erich[2]; Thompson, Addie[1]
[1] Department of Plant, Soil & Microbial Sciences; Michigan State University; East Lansing, MI 48824
[2] Department of Biochemistry and Molecular Biology; Michigan State University; East Lansing, MI 48824

Nitrogen fertilizer application is a necessary component to maximize maize productivity, but excessive usage leads to environmental and economic consequences. This problem is exacerbated by the fact that maize plants use only half of the nitrogenous inputs. Nitrogen use efficiency is a complex trait that is reliant on the plant's ability to assimilate and recycle N. Changes in gene expression are one mechanism maize has to react to N. It is generally understood how the maize transcriptome responds to N conditions during distinct stages of growth, flowering, and grain filling. However, little is known about how gene expression may drive variation between hybrids. Understanding how exactly different hybrids respond to N will greatly help breeding for improved sustainability. To characterize hybrid specific response to N, a panel of 5 hybrids was field grown in high and low N conditions. RNA sampling was done at the V7, V15, and R2 stages, while field-data was collected from accompanying plots. The 5 hybrids showed significant variation in their yield response to N. The component traits most associated with yield response were days to flowering and plant height. Transcriptomics reveals that the relative effect of N treatment is small at V7 but eclipses the effect of genotype by R2. Next, gene expression data was integrated with field phenotypes to identify which genes were associated with hybrid specific N response traits. A set of 370 genes that were most N responsive in the most yield responsive hybrid was generated based on differential expression test values and used for functional enrichment analysis. Notable gene ontology terms include protein folding chaperone, serine family amino acid biosynthesis, and the plastid cellular compartment. Taken together, these results can illustrate how hybrid-specific gene expression and accompanying traits help drive variation in yield response to N. Further analysis will integrate leaf and grain nutrient concentrations from the 5 hybrids to fill in important details about the leaf-grain source-sink relationship.

Funding acknowledgement: IMPACTS NRT, AgSpectrum Company

P84

GO-based comparative functional analysis of three maize genomes

(submitted by Blessing Ngara <ngarable@iastate.edu>)
Full Author List: Ngara, Blessing[1]; Fattel, Leila[1]; Yanarella, Colleen F.[1]; Kokulapalan, Wimalanathan[1]; Lawrence-Dill, Carolyn J.[1]
[1] Agriculture and Life Science Administration, Iowa State University, Ames, Iowa, United States, 50010

The Gene Ontology (GO) is a standard vocabulary arranged as a directed acyclic graph that is used to annotate and compute on functions of genes and their products. GOMAP, produces high-confidence, high coverage, and reproducible functional annotations for plants by combining multiple functional prediction approaches. We have annotated several plant genomes using the GOMAP pipeline, resulting in improved GO datasets for researchers' use. GOMAP has been continuously updated since it had been first published. Here, we demonstrate and describe the work to compare outputs of GOMAP with the 2017 OBO file to the 2022 OBO file to determine whether updating all crop plant gene function prediction datasets should be pursued. Maize (Zea mays ssp. mays) is the species for which our GOMAP datasets are most accessed and utilized. As such, we are using W22, PH207, and Mo17 as exemplars for this work.

Gene / Gene Models described: ; Zm00014a, Zm00008a,Zm00004b

Funding acknowledgement: National Science Foundation (NSF)

P85 @LeilaFattel

Gene ontology based comparative functional genomics in plants

(submitted by Leila Fattel <lfattel@iastate.edu>)
Full Author List: Fattel, Leila[1 2]; Yanarella, Colleen F.[2 3]; Wimalanathan, Kokulapalan[4 5]; Lawrence-Dill, Carolyn J.[1 2 3 4]
[1] Interdepartmental Genetics and Genomics, Iowa State University
[2] Department of Agronomy, Iowa State University
[3] Interdepartmental Bioinformatics and Computational Biology, Iowa State University
[4] Department of Genetics, Development and Cell Biology, Iowa State University
[5] Neuroscience Team, Atalanta Therapeutics, Boston

Better gene function predictions enable better hypotheses for testing gene functions via laboratory and field experiments, which saves time and resources. As more genomic sequences become available and the quality has become comparable, scientist are using genomic analyses to answer increasingly complex biological questions. Biologists have been using the genomic differences observed with comparative genomics to better understand the relationship between genes and phenotypes within and across species. We are interested in creating a proof of concept analytics software to help researchers access and use large-scale datasets to generate hypotheses and prioritize candidate genes for phenotypes of interest. Using GOMAP, we annotated gene function for several crop genomes. We recovered known evolutionary histories across plant species based on their functional annotation datasets. This indicates that the datasets across species retain sufficient biological signal to carry out and make use of GO for comparative functional genomics. As a next step, we are investigating the potential use of GO for comparative functional genomics within species to bridge the gap between gene function and phenotype for making biologically meaningful inferences and hypotheses. Here we focus on potential methods and ideas on how to analyze associations between genes and phenotypes using the NAM founder lines.

Funding acknowledgement: National Science Foundation (NSF), United States Department of Agriculture (USDA), IOW04714 Hatch Funding to Iowa State University

P86 @Yan_Geneticist

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

(submitted by Yan Zhou <yzhou86@iastate.edu>)
Full Author List: Zhou, Yan[1]; Kusmec, Aaron[1]; Schnable, Patrick S.[1]
[1] Department of Agronomy, Iowa State University, Ames, IA, 50011

The efficiency of canopy interception of solar radiation is a major contributor to the photosynthetic efficiency of crop plants. Light interception is itself a function of canopy architecture, including, leaf number, length, width, and angle, as well as azimuthal canopy orientation(s). We report on the ability of some maize genotypes to alter the orientations of their leaves during development in coordination with adjacent plants. Although these genotypes generally retain the typical alternate-distichous phyllotaxy of maize, their leaves grow parallel to those of adjacent plants of the same genotype. Previous morphological studies suggest this “parallel pattern” phenotype may be a response to the ratio of red to far-red light (R/FR), and hence a component of the shade avoidance syndrome (SAS). A genome-wide association study (GWAS) conducted on the parallel pattern phenotype identified candidate genes, many of which have been reported to be associated with SAS, including *phytochromeC2* (*phyC2*). In addition, GWAS on the fraction of photosynthetically active radiation (PAR) intercepted by canopies (designated “PAR interception”) of the diversity panel identified genes known to regulate leaf development, including *liguleless1* (*lg1*). Mutants in both the *phyC2* and *liguleless1* genes exhibit altered canopy patterns, such that the numbers of interrow leaves are greatly reduced as compared to non-mutant controls. This results in dramatically decreased PAR interception by the *lg1*, *lg2* and *Lg3* mutant canopies, demonstrating a novel function of these genes, which were originally defined by their ability to regulate ligule development. Consistent with the view that these alterations in canopy patterns are a component of SAS, our results demonstrate they are also influenced by plant density.

Gene / Gene Models described: *lg1, lg2, Lg3, phyC2*; GRMZM2G036297, GRMZM2G060216, GRMZM2G087741, GRMZM2G129889

P87

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

(submitted by Zhenyuan Lu <luj@cshl.edu>)
Full Author List: Lu, Zhenyuan[1]; Chougule, Kapeel[1]; Olson, Andrew[1]; Tello-Ruiz, Marcela K[1]; Wei, Sharon[1]; George, Nancy[2]; Papatheodorou, Irene[2]; Ware, Doreen[1 3]
[1] Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724
[2] EMBL-European Bioinformatics Institute, Wellcome Trust Genome Campus, Hinxton, CB10 1SD, UK
[3] USDA ARS NEA Plant Soil & Nutrition Laboratory Research Unit, Ithaca, NY, 14853, USA

The Gramene project has developed pan-genome subsites, as a natural extension of the Gramene and Ensembl infrastructures. Each pan-genome site is dedicated to the study of individual crop groups (e.g., maize, rice, sorghum, and grape). The Gramene project (http://www.gramene.org) was a key player in sequencing the first maize reference B73, more recently the 25 NAM founders, and continues to be engaged with the community. The maize pansite (https://maize-pangenome.gramene.org), established in 2021, hosts reference assemblies for 26 maize accessions. Each maize accession is hosted in a separate genome browser, providing access to gene-based views with entry points via text-based searches or through BLAST. The B73 V5 genome serves as the reference assembly for anchoring expression, population and pathway views; transcript abundance across a gene model at different tissues and stages of development and views of paralog expression support decision making on candidate gene selection. Phylogenetic analyses are based on maize-specific gene trees built from the assigned canonical protein for each gene locus. The protein-based trees allow users to rapidly traverse between the different maize accessions, where alleles are considered orthologs in the trees, as well as other species; copy-number variations within maize can also be evaluated alongside lineage-specific gene expansions. Protein homology is viewable as amino acid alignments and as gene neighborhood conservation. We adapted these views to deploy community curation tools–from the homology tab–for users to flag potential structural annotation issues. More recent work has used these gene trees to build a pan-gene index used as the inputs to improve and extend the previous gene structural annotation workflow. Release 3 will include updated annotations and access to the teosinte reference genome, expression, and Maize ENCODE (NSF-IOS-1445025) related studies. Funded by USDA-ARS-8062-21000-041-00D.

Funding acknowledgement: United States Department of Agriculture (USDA)

P88

Gramene: Comparative plant genome resource

(submitted by Doreen Ware <doreen.ware@usda.gov>)
Full Author List: Chougule, Kapeel[1]; Kumar, Vivek[1]; Kumari, Sunita[1]; Olson, Andrew[1]; Gladman, Nicholas[1 2]; Olson, Audra[1]; Tello-Ruiz, Marcela K[1]; Wei, Sharon[1]; Elser, Justin[3]; Gupta, Parul[3]; Naithani, Sushma[3]; George, Nancy[4]; Naamati, Guy[4]; D'Eustachio, Peter[5]; Dyer, Sarah[4]; Papatheodorou, Irene[4]; Jaiswal, Pankaj[4]; Ware, Doreen[1 2]
[1] Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 11724
[2] USDA ARS NEA Plant Soil & Nutrition Laboratory Research Unit, Ithaca, NY, 14853
[3] Dept Botany & Plant Pathology, Oregon State University, Corvallis, OR, 97331
[4] EMBL-European Bioinformatics Institute, Wellcome Trust Genome Campus, Hinxton, CB10 1SD, UK
[5] Dept Biochemistry & Molecular Pharmacology, NYU Grossman School of Medicine, New York, NY, 10016

The Gramene knowledgebase (www.gramene.org), established in 2002, was the first comparative plant genome database. Twenty one years later, the project has grown from hosting one crop genome to 128 plant genomes. Scaling the data has required collaborations, development of standards, dedication to FAIR principles. Gramene is built on public data available in archival resources and established standards, including software and workflows to provide added value and ease of data access. The current release 66 (December 2023) features 128 reference genomes, population data from 15 species, as well as expression data for 28 species. The genomes are inputs to the added value workflows, including whole-genome DNA alignments, and protein-based gene trees. The over 152K gene family trees provide insights on evolution and adaptation between eukaryotes, fly, yeast and worm, and expansion of the plant species from moss to flowering plants. We provide views to interrogate gene tree data, including amino acid alignments and gene neighborhood conservation, and have worked with different communities to curate the protein alignments to infer mis-annotations of gene structures. Expression information is available for multiple tissues and conditions and links to EBI-Atlas for more detailed information on each experiment. Where genetic variation data is available, SNPs are easily viewed and filtered based on their predicted functional effect (missense, stop-gained, etc.), impact (SIFT), and downloadable as images and tables. Genes can be queried via text-based searches and sequence-based BLAST. B73 accession serves as the maize reference assembly, and can be used to traverse to orthologs and paralogs for other species, enabling phylogenetic inference, insights of allelic genes within and across species, variation data, as well as lineage specific expansion and contraction, and synteny maps. We work with the community to coordinate data stewardship, training and feedback. Gramene is funded by USDA-ARS-8062-21000-041-00D.

Funding acknowledgement: United States Department of Agriculture (USDA)

P89

Growing up: How the UTR environment alters as genes age

(submitted by Colin Finnegan <cpf@iastate.edu>)
Full Author List: Finnegan, Colin[1]; Li, Jing[2]; Wurtele, Eve[3]; Vollbrecht, Erik[1]
[1] Iowa State University, 2288 Molecular Biology Building, 2437 Pammel Dr, Ames, IA, 50011
[2] TBD
[3] TBD

New genes are constantly emerging within the genome, with some surviving selection pressures to become established in populations. All old genes are survivors of this process, and the taxonomic level of the ancestral population in which they first appeared (hereafter called the phylostrata of the gene) can be used to date them. I have examined the UTRs of old genes and new genes and found that they differ in significant ways. Genes of different phylostrata have different densities of Mutator transposon insertion sites in their UTRs and different likelihoods of lacking UTRs entirely. I hypothesize that these differences can explain how new genes make the gradual transition to having all of the features recognizable in established genes.

Funding acknowledgement: United States Department of Agriculture (USDA)

P90

High throughput phenotyping of field excavated roots for diverse maize inbred and hybrid lines under different N conditions.

(submitted by Musa Ulutas <ulutas.musa@huskers.unl.edu>)
Full Author List: Ulutas, Musa[1 2]; Delen, Yavuz[1]; Ge, Yufeng[2 3]; Yang, Jinliang[1 2]
[1] Department of Agronomy and Horticulture, University of Nebraska-Lincoln, Lincoln, NE 68583, USA
[2] Center for Plant Science Innovation, University of Nebraska-Lincoln, Lincoln, NE 68583, USA
[3] Department of Biological Systems Engineering, University of Nebraska-Lincoln, Lincoln, NE 68583, USA

Maize (*Zea mays* ssp. Mays) is a crucial cereal crop that supports global food security. As the climate changes, nitrogen (N) use efficiency becomes increasingly important for maize production without compromising yield. Image-based high-throughput phenotyping is a vital tool in this effort, as it allows for accurate and efficient phenotypic measurement in a cost-effective manner. While many studies have focused on aboveground traits, belowground root-related traits have not been extensively studied. To investigate the root architecture and phenotypic properties (number of aerial roots, stem diameter, internode length, and fresh root weight), we collected a set of BGEM lines (n = 304 inbred and n = 197 hybrids) planted on the field in low and high N conditions. The root samples of n = 2,100 plants were collected, and the soil around the roots was washed out for automated image-based phenotyping using the automated conveyor belt LemnaTec system. Results showed a high variation in root structure, stem diameter, number of aerial roots, and internode length among genotypes. This study's root phenotyping pipeline and traits extracted from the images will enhance our understanding of root biology and facilitate breeding for below-ground root traits, which are important for improving nitrogen-use efficiency in maize and mitigating the negative effects of climate change.

Funding acknowledgement: United States Department of Agriculture (USDA)

P91

High-throughput phenotypic analysis of plant growth in lunar regolith simulant and the potential applications in space agriculture

(submitted by Cassandra Palmer <cpalmer9@unl.edu>)
Full Author List: Palmer, Cassandra S.[1 2]; Stoerger, Vincent[2 4]; Yang, Jinliang[1 2]; Ge, Yufeng[3]
[1] Department of Agronomy and Horticulture, University of Nebraska-Lincoln; Lincoln, NE 68588, USA
[2] Center for Plant Science Innovation, University of Nebraska-Lincoln, Lincoln, NE 68583, USA
[3] Department of Biological Systems Engineering, University of Nebraska-Lincoln, Lincoln, NE 68583, USA
[4] Agricultural Research Division, University of Nebraska-Lincoln, Lincoln, NE 68583, USA

The potential for human habitation on extraterrestrial bodies depends on efficient use of in situ resources, particularly for space agriculture and crop production. In an extraterrestrial human habitation, plants will play major roles in maintaining climate, recycling carbon dioxide to produce oxygen, and providing carbohydrates, proteins, dietary fatty acids, and micronutrients. An experiment was conducted in a greenhouse to evaluate the performance of soybean plants in a lunar mare regolith simulant (LMS-1). A multi-omics approach was employed, where high-throughput phenomics with multiple imaging modules was used to characterize plant growth and development. The development of soybean plants in LMS-1 was slowed, and they expressed stress-related phenotypic responses as examined using image analysis software. RNA sequencing (RNA-Seq) and transcriptomic analysis will be used to validate the data collected from the high-throughput plant phenotyping. The plants' phenotypic and transcriptomic responses identified in this study, because of stresses from LMS-1, will provide insight for engineering approaches that will best support space agriculture and crop production on the Moon and Mars. In the further, other crops, such as maize, will be tested with the developed remote sensing and non-destructive technologies for space agriculture research and production.

P92 @6zongyan

Identification of seed storage proteins across andropogoneae genomes with machine learning

(submitted by Zong-Yan Liu <zl843@cornell.edu>)
Full Author List: Liu, Zong-Yan[1]; Zhai, Jingjing[2]; Buckler, Edward[1 3]
[1] Section of Plant Breeding and Genetics; Cornell University; Ithaca, NY USA 14853
[2] Institute for Genomic Diversity; Cornell University; Ithaca, NY USA 14853
[3] USDA-ARS; Ithaca, NY, USA 14853

Seed storage proteins not only function as a source of nitrogen and sulfur for developing seedlings but also offer food proteins for humans and animals. These proteins are typically present in the specialized storage tissues of plants and particularly in the kernels of cereal crops such as wheat and maize. In maize, more than 60% of the total storage proteins in the kernels are zein, which is a family of plant storage proteins with a high proline amino acid content. Zeins have been identified and functionally investigated, but it is still not clear how many storage proteins exist in other plant species. Further studies are essential to get a better understanding of the composition of storage proteins and to uncover any other storage proteins that have not been previously identified. Here, the physicochemical properties of seed storage proteins in the UniProt database were studied, which indicated that these proteins shared similar characteristics and were more uncharged, unstable, and flexible compared to all other non-storage proteins. Utilizing the compatible features, we employ a language modeling tool named ProtTrans to translate all the seed storage proteins in the database into embedding features. With these protein features, we are able to distinguish seed storage proteins from non-storage proteins based on the distance between their features. Because the storage proteins have similar characteristics, we created a support vector machine classifier with good accuracy across all the plant seed storage proteins. Using this model, we plan to scan across all available Andropogoneae genomes and search for new seed or vegetative storage proteins that have not been explored.

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

P93

Improving sensitivity of variant effect estimates on RNA expression through cross-species training

(submitted by Travis Wrightsman <tw493@cornell.edu>)
Full Author List: Wrightsman, Travis[1]; Romay, M Cinta[2]; AuBuchon-Elder, Taylor[3]; Phillips, Alyssa[4]; Syring, Michael[5]; Kellogg, Elizabeth A.[3]; Marand, Alexandre P.[6]; Buckler, Edward S.[1 2 7]
[1] Section of Plant Breeding and Genetics; Cornell University; Ithaca, NY, USA 14853
[2] Institute for Genomic Diversity; Cornell University; Ithaca, NY, USA 14853
[3] Donald Danforth Plant Science Center; St. Louis, MO, USA 63132
[4] Department of Evolution and Ecology; University of California, Davis; Davis, CA, USA 95616
[5] Iowa State University; Ames, IA, USA 50011
[6] Department of Genetics; University of Georgia; Athens, GA, USA 30602
[7] Agricultural Research Service; United States Department of Agriculture; Ithaca, NY, USA 14853

Targeted genome editing technologies have the potential to accelerate breeding pipelines in any species but require prior knowledge of which loci to change and what changes to make. One promising approach for identifying useful editing targets is through interpretation of sequence-based deep learning models trained on biological targets such as RNA expression. Since deep learning models require large and diverse training sets, which are rarely available within a single species, we chose to leverage data from multiple grass species to train RNA expression models on larger sample sizes. Millions of years of evolution within the grasses has not only resulted in a large diverse pool of functional alleles to train with but also a strong signal of purifying selection on functional loci. We used Salmon to get transcript-specific quantifications of RNA-seq datasets from around 40 grass species with annotated genome assemblies. We then extracted regulatory sequences near each transcript model as well as nearby accessible regions predicted by our previous model, a2z, as input features. These features were used to train a few different model architectures, including a recurrent convolutional network and a transformer, that predicted RNA expression level from regulatory sequence. We saw differences in performance across architectures, with each architecture capable to some degree of predicting expression from sequence across species. Next, we will apply interpretation methods to these models to rank alleles in maize populations by effect size and pursue new biological insights into the gene regulatory grammar of grasses.

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

P94 @hwb333

MNase sequence bias fails to predict MOA-seq chromatin profiling peaks

(submitted by Zehta Glover <zglover@fsu.edu>)
Full Author List: Glover, Zehta S.[1]; Singh, Ayush[1]; Hanley-Bowdoin, Linda K.[2]; Bass, Hank W.[1]
[1] Department of Biological Science, Florida State University, Tallahassee, FL, USA, 32303
[2] Plant and Microbial Biology, North Carolina State University, Raleigh, NC, USA, 27695

Micrococcal nuclease (MNase, E.C. 3.1.31.1) is an enzyme that is commonly used to probe chromatin structure and gene regulation in eukaryotic cells. However, MNase has a known preference for AT-rich sequences, which can lead to complications in data interpretation. To address this problem, we performed control digests using purified, non-chromatin DNA from maize B73 earshoots. Next-generation DNA sequencing libraries from these samples were used for genome alignment and read coverage MACS3-based peak calling. Replicate peak numbers ranged from 69,000 to 141,000. These peaks were used for comparison with peaks from MNase-based cistrome profiling from maize (103,000 MOA-seq peaks from Savadel et al., PLoS Genetics, 2021). Remarkably, Only 0.9% to 1.9% of the chromatin MOA-seq peak base pairs were shared with the purified DNA control peaks. We also carried out base frequency analysis to check for the expected local increase in percent AT surrounding the edges of the aligned fragments. We did observe this increase, but the peak intersection results suggest that this property does not solely predict chromatin profiling signals. These data indicate that the known sequence bias of MNase does not significantly affect the use of MNase to map candidate cis-element occupancy, while providing valuable control datasets for any past or future MNase hypersensitive B73 chromatin profiling assays.

Funding acknowledgement: National Science Foundation (NSF)

P95

Maize gene expression responses to late season application of various nitrogen sources

(submitted by Alexandria Tran <tran30@illinois.edu>)
Full Author List: Tran, Alexandria[1]; Moose, Stephen[1]; Marshall-Colon, Amy[1]
[1] University of Illinois, Urbana-Champaign, Illinois, United States 61801

There are many benefits to late season nitrogen (N) fertilization, including the reduction of N loss from leaching as well as more efficient use of N post silking for improved grain yield. The plant's ability to use N may vary based on the source, ammonia or nitrate. Previous work has shown no change to ear weight with application of nitrate after V12, but ammonia has been shown to improve ear weight when applied as late as V15. Working with Gaspe flint, a FastCorn line, in a hydroponics setup, we are able to observe changes in gene expression via RNA-sequencing after pulses of each N source. We can then evaluate responses from a systemic perspective by sampling across three tissues–the roots, shoots, and immature ears–and compare them between the two N sources. With both likelihood ratio testing and gene regulatory network construction, several known differentially expressed genes including asparagine synthetase, CCA1, and NACTF108 were reconfirmed and others, including transcription factors, have been identified as future targets for study.

Gene / Gene Models described: *nactf108, cca1, asn4*; Zm00001eb135910, Zm00001eb172450, Zm00001eb396990
Funding acknowledgement: Foundation for Food and Agriculture Research

P96

Maize orphan genes and their potential association with cuticle synthesis

(submitted by Yuxiang Guo <yxguo@iastate.edu>)
Full Author List: Guo, Yuxiang[1 2]; Chen, Keting[1]; Li, Jing[1]; Eckhoff, Emily[1 3]; Bond, Darcy[1 4]; Wurtele, Eve Syrkin[1 2]; Yandeau-Nelson, Marna D.[1 2 3]
[1] Department of Genetics, Development & Cell Biology, Iowa State University; Ames, Iowa, 50010
[2] Interdepartmental Bioinformatics and Computational Biology Graduate Program, Iowa State University; Ames, Iowa, 50010
[3] Undergraduate Bioinformatics and Computational Major, Iowa State University; Ames, Iowa, 50010
[4] Horticulture Undergraduate Major, Iowa State University; Ames, Iowa, 50010

Orphan genes are genes encoding species-specific proteins that do not share orthology to any other annotated genes in the biosphere. Recent studies on the functionality of orphan genes demonstrate their impact on the development and metabolism of an organism. For example, the dau1 orphan found in nematodes controls larval development according to the copy number variations among different strains (Mayer et al., 2015). In plants, the QQS (Qua-Quine Starch) orphan, unique to Arabidopsis, modulates carbon and nitrogen allocation (Li et al., 2015). In maize, approximately 39,000 orphan transcripts have been identified from publicly available maize transcriptome data, however, their potential functions are unknown. The goal of this work is to identify orphan genes that are associated with plant cuticle development, particularly in the reproductive silks. The hydrophobic cuticle covers the plant epidermis and protects the plants against stressful environments. It is composed of a cutin polyester matrix and cuticular waxes that are infused within or laid on top of the cutin matrix.
In this study, we identified putative silk-specific orphan genes via a phylostratigraphy approach (Arendsee et al., 2019), examined differential expression of the orphan genes along the silk length and between B73 and Mo17 inbred lines, and queried potential associations between these orphans and the cuticular waxes. We discovered significant expression differences of silk-related orphan genes between husk-encased and emerged portions of the silks, and have identified interesting correlations between orphan gene expression and cuticle composition. These discoveries will pave the way toward the broader understanding of the functions of orphan genes and the genetic networks that underlie important metabolic pathways.

Funding acknowledgement: National Science Foundation (NSF)

P97

Mapping and functional characterization of cis-regulatory variation in maize

(submitted by Andrea Gallavotti <agallavotti@waksman.rutgers.edu>)
Full Author List: Gallavotti, Andrea[1 2]; Chen, Zongliang[1]; Li, Miaomiao[3]; Huang, Carol S.[3]; Schmitz, Robert J.[4]; Galli, Mary[1]
[1] Waksman Institute of Microbiology, Rutgers University, Piscataway, NJ, 08854, USA
[2] Department of Plant Biology, Rutgers University, New Brunswick, NJ, 08901, USA
[3] Department of Biology, New York University, New York, NY, 10003, USA
[4] Department of Genetics, University of Georgia, Athens, GA, 30602, USA

The domestication and improvement of many plant species has frequently involved modulation of transcriptional outputs and continues to offer much promise for targeted trait engineering. In large crop genomes such as maize regulatory regions constitute only a small fraction of the total genomic space and remain poorly annotated. Transcription factors (TFs) bind to short DNA sequence motifs in regulatory regions of target genes and control the gene expression changes responsible for plant developmental programs and environmental responses. While potential TF binding sites are naturally abundant within a genome, only a very small fraction of sites are actually bound by TFs and able to affect expression of nearby genes. Using a combination of DAP-seq and ATAC-seq for detecting TF binding and open chromatin regions, respectively, we are generating large-scale cis-regulatory module (CRM) maps in two different maize inbred lines, B73 and Mo17. In addition, many TFs do not work in isolation but can form homo and heterodimers and interact with other proteins that can alter their DNA binding activity. To understand how plant TF heterodimerization alters TF DNA binding at the genome-wide level we developed the doubleDAP assay. We are currently using this assay to probe differences in binding potential of certain bHLH heterodimers involved in maize developmental processes including inflorescence development (BARREN STALK1) and stomata formation (ZmSPCH, ZmMUTE and ZmFAMA). Our findings reveal that relative to their homodimeric counterparts, certain heterodimeric combinations show unique binding properties. Our overall goal is to identify functional CRMs and to understand 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)

P98 @ally_schumacher

Multi-omic analyses of maize color and leaf traits

(submitted by Ally Schumacher <schum193@msu.edu>)
Full Author List: Schumacher, Ally[1]; Sitar, Sidney[1]; Durst, Leah[1]; Ji, Zhongjie[1]; Shrote, Robert[1]; Newton, Linsey[1]; Schnable, James[2]; Thompson, Addie[1]
[1] Department of Plant, Soil & Microbial Sciences; Michigan State University; East Lansing, MI 48824
[2] Center for Plant Science Innovation and Department of Agronomy and Horticulture, University of Nebraska-Lincoln, Lincoln, NE 68588

Maize breeding pipelines commonly rely on the efficient and accurate estimation of phenotypic traits or breeding values from different data types, including genomics, image or sensor based phenomic features, or other multi-omic datasets. The efficacy of different data types will vary for different trait architectures and populations. Beyond increasing model accuracy, it is possible that combining different feature types into machine learning models will enable us to make novel marker-trait, feature-trait, or even marker-feature discoveries, increasing our understanding of genetic control of biochemical pathways. Using data collected from the Wisconsin Diversity Panel grown in Michigan field seasons 2020-2021, we will generate novel prediction models incorporating different -omics data types. We will then compare the accuracy of models and explanatory contribution of features derived from (i) drone-based field imagery, (ii) genetic markers (SNPs), and (iii) phenolic compound accumulation to predict phenotypic traits - primarily silk, anther, or kernel color and specific leaf weight. Genes known to lead to changes in color and phenolic compounds can be used directly as predictors (fixed effects in modeling other traits), but conversely can be used as traits themselves (PheWAS) to identify other features that indicate allelic status at those loci. Ultimately, machine learning models will predict phenotypic trait values while quantifying the impact of different data types on predictive performance and identifying phenomic features or additional candidate genes of interest to be used in future investigations.

Funding acknowledgement: National Science Foundation (NSF), IMPACTS NRT

P99 @CFYanarella

Proof of concept for spoken natural language descriptions of phenotypes for association genetics applications

(submitted by Colleen Yanarella <cfy@iastate.edu>)
Full Author List: Yanarella, Colleen F.[1 2]; Fattel, Leila[2 3]; Kristmundsdóttir, Ásrún Ý.[4]; Lawrence-Dill, Carolyn J.[1 2 3 4 5]
[1] Interdepartmental Bioinformatics and Computational Biology, Iowa State University, Ames, IA
[2] Department of Agronomy, Iowa State University, Ames, IA
[3] Interdepartmental Genetics and Genomics, Iowa State University, Ames, IA
[4] College of Agriculture and Life Sciences, Iowa State University, Ames, IA
[5] Department of Genetics, Development, and Cell Biology, Iowa State University, Ames, IA

Imagine walking through a field and saying aloud what you see, then using that audio file to conduct an association study. Advancements in Natural Language Processing (NLP) have allowed for processing large volumes of descriptive data. To make these advancements applicable for biologists interested in better understanding phenotypes and traits, we are developing methods to collect and process in-field descriptions of maize using recordings of spoken phenotype descriptions. We planted the Wisconsin Diversity panel in Boone, Iowa (summer of 2021). Nine undergraduate student workers recorded spoken descriptions of the Wisconsin Diversity panel lines in the field. We instructed the students to describe certain plant parts and other miscellaneous attributes using their own words. For comparative purposes, we also collected numerically scored phenotypes using traditional data collection techniques and images of each line. Our pipeline for processing the hundreds of hours of spoken descriptive data starts with the Amazon Web Services (AWS) cost-effective transcription service Transcribe. We can compute on the text descriptions of plants produced by Transcribe using NLP tools, which enables us to generate the input for tools that are commonly used by the maize research community to perform Genome-Wide Association Studies (GWAS). The results of the association studies completed using spoken data are then compared to published associations. Generating spoken descriptive datasets and protocols for demonstrating biologically relevant approaches for these data are anticipated to enable the maize research community to use innovations in language processing for in-field phenotyping methods.

Funding acknowledgement: National Science Foundation (NSF), United States Department of Agriculture (USDA), Iowa State University Plant Sciences Institute, P3 NSF Research Traineeship DGE#1545463, AIIRA NSF & USDA NIFA Award #2021-67021-35329, IOW04714 Hatch Funding to Iowa State University

P100

Regulatory networks governing nitrogen use efficiency in maize and sorghum

(submitted by Janeen Braynen <braynen@cshl.edu>)
Full Author List: Braynen, Janeen[1]; Zhang, Lifang[1]; Kumari, Sunita[1]; Kumar, Vivek[1]; Olson, Andrew[1]; Regulski, Michael[1]; Liseron-Monfils, Christophe[2]; Gaudinier, Allison[3]; Frank, Mary[4]; Shen, Bo[4]; Kochian, Leon[5]; Brady, Siobhan[6]; Ware, Doreen[1 7]
[1] Cold Spring Harbor Laboratory, 1 Bungtown Road, Cold Spring Harbor, NY 11724.
[2] National Research Council Canada / Conseil national de recherches Canada, 1200 Montreal Road Ottawa, Ontario K1A 0R6, CA.
[3] Miller Institute University of California, Berkeley, CA 94720
[4] Corteva, Agriscience
[5] University of Saskatchewan, Saskatoon, SK S7N4L8, CA
[6] Department of Plant Biology and Genome Center, UC Davis, Davis, CA, 95616
[7] USDA-ARS-NAA, Ithaca, NY 1485

Regulation of nitrogen availability is vital to increase crop production and reduce negative impacts on the environment. Previous studies of nitrogen availability under limiting conditions in Arabidopsis used an enhanced yeast-one hybrid assay (Y1H) gene regulatory network (GRN) to understand the response to nitrogen. However, the early response of nitrogen availability using a Y1H GRN network in crop species, such as maize is unknown. Here, we constructed a yeast one hybrid GRN for NUE in maize and used temporal expression patterns to profile the early response to nitrogen limitation and recovery. Key transcription factors (TFs) that regulate maize genes were involved in NUE with 1625 protein-DNA-interaction (PDI). Furthermore, 35% of the interactions in the network were conserved among various nitrogen-related processes between Arabidopsis and maize. Among such, three TF families were over-represented, including ERF, bZIP, and MYB. To assess if such regulated interaction is conserved in other crop species, we used the Gramene compara database to obtain orthologs to project the NUE network from maize to sorghum. The NUE networks identified here can provide critical insight into the early response to nitrogen regulation for agronomically important crops.
This work is funded by the US Department of Agriculture, Agriculture Research Service under Award Number 8062-21000-041-00D.

Funding acknowledgement: United States Department of Agriculture (USDA)

P101 @1amandagilbert

Structural and functional properties of core and dispensable genes in maize

(submitted by Amanda Gilbert <agilber@umn.edu>)
Full Author List: Gilbert, Amanda M[1]; Qiu, Yinjie[1]; Hirsch, Candice N[1]
[1] University of Minnesota

The core genome describes the set of genes shared by all sequenced individuals, while the dispensable genome refers to the sequences shared by only a subset of individuals. The pan-genome, consisting of the core and dispensable genes, represents the full complement of genetic material present in a species. Previous pan-genome analysis of the 26 maize NAM founder lines identified 103,033 pan-genes; 32,052 genes belong to the core and near-core fractions, and 71,486 to the dispensable and private fractions. Here we further assess the structural and functional properties of core and dispensable genes. Core genes exhibit greater complexity, as indicated by significantly higher exon count of the canonical gene models and higher isoforms number per gene, and the percent total GC content of the coding sequence region in core genes is higher than in dispensable genes. Analysis across evolutionary time shows core and dispensable genes are more similar in exon, intron, and GC content at younger phylostrata levels. Additionally, dispensable genes that are syntenic to sorghum share more similar properties to core genes across all phylostra levels.This study provides a deeper understanding of the functional properties of different classes of genes and insight into maize genome evolution.

Funding acknowledgement: National Science Foundation (NSF)

P102

Systematic exploration of transcription factor function in maize

(submitted by Taylor Strayhorn <taylor.strayhorn@uga.edu>)
Full Author List: Strayhorn, Taylor[1]; Birla, Aditya[1]; Anthony, Michener[1]; Freeman, Grant[1]; Johanson, Ben[1]; Nelms, Brad[1]
[1] University of Georgia, Athens, Georgia, 30602

Transcription factors (TFs) play an important role in many processes in plants by inducing reproducible and distinct responses. In Maize, there are over 3000 TFs and we don't know the function of many of those. When TFs are expressed ectopically, their native pathways can be induced. Our goal is to test TF function large scale through ectopic expression. We have established methods to transform leaf protoplasts with individual plasmids containing TFs that achieve a median transformation efficiency of 70%. The immediate transcriptome-wide response induced by each TF will be measured by RNA sequencing. In a pilot study, we overexpressed 140 TFs from the maize TFome, a collection of cloned maize TF plasmids, with 30% of TFs providing clear, distinct, and reproducible responses. In the next steps, we will continue the established workflow through the remainder of the TFome producing a resource for the maize community.

P103

The association of indels with meiotic recombination sites in maize

(submitted by Nikita Sajai <ns623@cornell.edu>)
Full Author List: Sajai, Nikita[1]; Pawlowski, Wojtek[1]; Epstein, Ruth[1]; Wang, Minghui[1]
[1] Cornell University; Ithaca, NY, USA 14850

The processes occurring during meiotic recombination, from the initiation of DNA double-strand breaks (DSBs) to the completion of crossing-over (CO) formation, have numerous opportunities for inaccuracy. In particular, several types of missteps during recombination, including the formation of double DSBs, could lead to genome deletions. To explore this phenomenon in maize, we examined the occurrence of indels at recombination sites. To do it, we mapped indels to meiotic DSB hotspots and CO sites and measured the presence and degree of enrichment of indels of different sizes. We assessed the indel generation potential of meiotic recombination by calculating three measures: indel overlap by recombination sites, recombination site overlap by indels, and indel density at recombination sites. We found substantial enrichment of small indels (1-50 bp) at CO sites, providing the first strong evidence of mutagenicity of meiotic recombination in plants. Small indel density decreased in regions 2 kb upstream and downstream from the recombination sites, implying that the indels were generated in a localized fashion. Indels have the potential to alter gene function and plant phenotype, marking their importance as a source of genetic diversity. Thus. understanding mechanisms of indel origination and their relationship to the recombination mechanism is important for efforts that exploit genetic diversity for crop improvement.

Funding acknowledgement: National Science Foundation (NSF)

P104 @prasanth_jyothi

The case for retaining natural language descriptions of phenotypes in plant databases and a web application as proof of concept

(submitted by Jyothi Prasanth Durairaj Rajeswari <jyothi@iastate.edu>)
Full Author List: Braun, Ian R.[1 2]; Yanarella, Colleen F.[1 2]; Durairaj Rajeswari, Jyothi Prasanth[3]; Bassham, Diane C.[4]; Lawrence-Dill, Carolyn J.[1 2 4 5]
[1] Interdepartmental Bioinformatics and Computational Biology, Iowa State University
[2] Department of Agronomy, Iowa State University
[3] Department of Electrical and Computer Engineering, Iowa State University
[4] Department of Genetics, Development and Cell Biology, Iowa State University
[5] Interdepartmental Genetics and Genomics, Iowa State University

Motivation: Finding similarity across phenotypic descriptions is not straightforward, with previous successes in computation requiring significant expert data curation. Natural language processing of free-text phenotype descriptions is often easier to apply than intensive curation. It is therefore critical to understand the extent to which these techniques can be used to organize and analyze biological datasets and enable biological discoveries.
Results: A wide variety of approaches from the natural language processing domain perform as well as similarity metrics over curated annotations for predicting shared phenotypes. These approaches also show promise both for helping curators organize and work through large datasets as well as for enabling researchers to explore relationships among available phenotype descriptions. Here we generate networks of phenotype similarity and share a web application, QuOATS (Querying with Ontology Annotations and Text Similarity), for interrogating a dataset of associated plant genes using these text mining approaches. The phenotypic similarity is identified by a text-based approach (translating phenotypic descriptions to vectors using BERT, BioBERT, Word2Vec, and Doc2Vec models and finding similarity using cosine similarity) and a curation-based approach (finding similarity from GO terms, PO terms, or EQ statements). Example situations and species for which the application of these techniques is most useful are discussed.Availability: The dataset used in this work is available at https://git.io/JTutQ. The code for the analysis performed here is available at https://git.io/JTutN and https://git.io/JTuqv. The code for the web application discussed here is available at https://git.io/Jtv9J, and the application itself is available at https://quoats.dill-picl.org/.

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

P105
Understanding the role of *ZmWUS1* and cis-regulatory elements in maize inflorescence development at single-cell resolution

(submitted by Sohyun Bang <Sohyun.Bang@uga.edu>)
Full Author List: Bang, Sohyun[1]; Zhang, Xuan[2]; Gregory, Jason[3]; Chen, Zongliang[3]; Gallavotti, Andrea[3]; Schmitz, Robert[2]
[1] Institute of Bioinformatics, University of Georgia, Athens, GA, 30602, USA
[2] Department of Genetics, University of Georgia, Athens, GA 30602, USA
[3] Waksman Institute of Microbiology, Rutgers University, Piscataway, NJ, USA

Maize inflorescences arise through cell divisions from a group of undifferentiated stem cells within the inflorescence meristem. A gene regulatory network in the inflorescence meristem controls these cell divisions, shaping the structure of the ear. In Arabidopsis, the organization of stem cells is maintained in the organizing center of meristems by *WUSCHEL (WUS)*. *WUS* encodes a transcription factor whose expression is regulated by a negative feedback loop with *CLAVATA3* in the central zone. The *Barren inflorescence3* (*Bif3)* mutant in maize is caused by a tandem duplicated copy of *ZmWUS1*, a maize co-ortholog of *WUS*, and it displays enlarged inflorescences, whereas the number of spikelet pair meristems is reduced. We hypothesized that the duplicated copy of *ZmWUS1* in *Bif3* causes alterations of gene expression by changing chromatin accessibility at target genes in both a cell autonomous and non-cell-autonomous manner. We performed single cell Assay for Transposase-Accessible Chromatin followed by sequencing (scATAC-seq) in early developing ears in wild type and *Bif3* mutants to investigate potential *ZmWUS1* targets, including their cis-regulatory elements at single-cell resolution. We identified the same composition of cell types in both mutant and wild type, however, we observed the chromatin accessibility patterns in the organizing center cells, where *ZmWUS1* is expressed, are quite diverse between *Bif3* and wild type. We are currently investigating the cell autonomous effect of *ZmWUS1* within the organizing center by comparing cis-regulatory elements within differential accessible chromatin regions between wild type and *Bif3* mutants. We are also analyzing the chromatin accessibility patterns in other cell types within the inflorescence tissue to identify direct targets of ZmWUS1 and cis-regulatory elements that have important roles in the developing maize inflorescence.

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

P106 @SidneySitar
Utilizing FT-MIR spectroscopy and LC-MS to model phenolic compound accumulation in diverse maize kernels

(submitted by Sidney Sitar <sitarsid@msu.edu>)
Full Author List: Sitar, Sidney[1]; Gomez-Cano, Lina[2]; Sherif, Faisal[1]; Miesel, Jessica[1]; Grotewold, Erich[2]; Thompson, Addie[1]
[1] Department of Plant, Soil & Microbial Sciences; Michigan State University; East Lansing, MI 48824
[2] Department of Biochemistry and Molecular Biology; Michigan State University; East Lansing, MI 48824

Phenolic compounds are chemically identified as compounds containing both an aromatic ring and a hydroxyl group, where the hydroxyl group is attached directly to the aryl group. They are phytochemicals that are distributed throughout plant tissues and are considered specialized metabolites synthesized through the pentose phosphate, shikimic acid and phenylpropanoid pathways. Phenolic compounds, including phenylpropanoids and flavonoids, retain bioactive properties that provide beneficial nutritional health effects by those who consume them and provide important roles in the plant defense pathways and the plant immune system. In this study, maize kernels from a subset of lines in the Wisconsin Diversity Panel underwent phenolic compound extraction with aqueous methanol. After extraction, detection and quantification were performed using liquid chromatography - tandem mass spectrometry (LC-MS/MS). Because this method is time consuming and costly, discovering a faster way to determine the phenolic content of kernel tissue is essential for creating a more accessible means of data collection that can be used to influence breeding decisions. Using the remnant ground kernel tissue, samples were analyzed by Fourier-transform mid-infrared (FT-MIR) spectroscopy, allowing the exploration and modeling of the spectral reflectance in combination with the analytical chemistry. Exploring a variety of spectral preprocessing methods that are commonly used in determining soil content profiles allowed us to inspect the varying levels of prediction accuracy of the models. Creating an accurate modeling technique based upon the spectroscopy results will provide an insightful and more accessible tool for breeders to input phenolic compound information into their programs, without the costly analytical chemistry.

Funding acknowledgement: United States Department of Agriculture (USDA)

P107

reelProtein: filtering functional protein annotations with machine learning

(submitted by Jingjing Zhai <jz963@cornell.edu>)
Full Author List: Zhai, Jingjing[1]; Schulz, Aimee J[2]; Shu, Sheng-Kai[1]; Romay, M Cinta[1 2]; Buckler, Edward S[1 2 3]
[1] Institute for Genomic Diversity, Cornell University, Ithaca, NY USA 14853
[2] Section of Plant Breeding and Genetics, Cornell University, Ithaca, NY USA 14853
[3] USDA-ARS; Ithaca, NY, USA 14853

Rapidly evolving pan-genomes enable us to identify all possible gene models within a species, but it is still uncertain which gene model is correct and leads to a functional protein. The maize pan genome made up of the 26 NAM founders consists of 103,033 pan-genes, of which only 27,910 are core genes. Core genes are more likely to be correctly annotated and translated because they are present in all individual pan-genome taxa, and also are considered as more functionally conserved. However, generating pan-genomes for all species is impractical, therefore computational methods to identify core genes are necessary and cost-effective. Toward this goal, we developed the reelProtein pipeline, an extension of the reelGene pipeline, which consists of an XGBoost model to classify core and non-core proteins. To fully characterize each protein, we leveraged UniRep, a deep representation learning model trained on 27 million proteins from UniProt, to learn the structural and evolutionary information of each protein. Each protein was characterized as a 256-dimensional vector which was used for training the XGBoost model. We applied reelProtein on maize B73 gene models and found reelProtein achieved high prediction performance with an AUC of 0.833 and PR-AUC of 0.792. In the maize proteome, we can recover 80.4% maize core proteins, while 31.7% dispensable (non-core) proteins are also predicted as core proteins. These results indicated that the reelProtein pipeline could be a promising way to identify core proteins across maize and other grass genomes.

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

P108 @Citogenetica

A new B chromosome repeat and its relationship to other repetitive sequences of maize

(submitted by Mateus Mondin <mmondin@usp.br>)
Full Author List: Costa, Gabriel H. S.[1]; Silva, Gabriel F.[1]; Birchler, James A.[2]; Mondin, Mateus[1]
[1] ESALQ, University of São Paulo; Piracicaba, SP, Brazil, 13418-900
[2] Division of Biological Sciences, University of Missouri; Columbia, MO 65211, USA

The B chromosome is an interesting source to study the repetitive fraction of the maize genome. Recently the whole sequencing and assembly of the maize B-chromosome were published. Using this B chromosome assembly, we walked across the entire chromosome, collecting sequences differing from those known and described so far. A sample of 15 repeats interspersed among K180 and showed a high homology level was identified. The consensus sequence exhibited some level of similarities to a region at chromosome 4 intermingled with Cent-4 and near Cent-C. An analysis of this region into chromosome 4 shows a series of short repeats with a high level of homology. A total of 490 repeats were identified in this region. These two new repetitive sequences did not show any level of homology to K180 and Cent-C. The sequences isolated from B-chromosome were named B_ola_1, which has 189 bp. A comparative analysis among B-specific repeat, Cent-4 and B_ola_1, and B_ola_2 shows they shared a small conserved region. A comparison between the B-specific repeat with B_ola_1 shows two main areas of homology encompassing 43 and 79 bp, respectively. These two regions are in the second half of the B_specific repeat. B_ola_2 has homology to a central part of the Cent-4 repeat. However, this same region is less conserved when Cent-4 is compared to B_ola_1, but it is possible to identify its position clearly. A careful analysis of this conserved region shows a connection between these two new repetitive sequences identified with Cent-4 and B-specific repeats. A complete survey of the repetitive sequences of the B chromosome and its comparative analysis with the maize genome might help elucidate the paths of its origin.

P109

Cytological evidence supports the two-compartment model of maize euchromatin distinguished by replication timing

(submitted by Hafiza Sara Akram <ha20be@fsu.edu>)
Full Author List: Akram, H. Sara[1]; Ruiz, Maia L.[1]; Wear, Emily E.[2]; Mickelson-Young, Leigh A.[2]; Thompson, William F.[2]; Hanley-Bowdoin, Linda K.[2]; Bass, Hank W.[1]
[1] Department of Biological Science, Florida State University, Tallahassee, FL, USA, 32303
[2] Plant and Microbial Biology, North Carolina State University, Raleigh, NC, USA, 27695

Our overall project aims to understand the genomic and spatial organization of DNA replication in maize by varieties (B73 and NC350) and sorghum. Replication timing (RT) is a regulated process that ensures the genome and epigenome is completely and coordinately replicated during S-phase. Previously, we found that genomic regions that replicate in early versus middle S phase could be cytologically distinguished by two variables, replication timing and DAPI intensity. This led to our working hypothesis that euchromatin in maize exists in two intermingled but spatially distinct compartments (the "mini-domain model" from Bass et al., Plant Mol Biol, 2015. These spatially distinct compartments may also be present at other stages of the cell cycle such as G1. To test this idea, we plan to use two complementary assays, one cytological (3D oligo painting FISH) and one molecular (HiC). For the 3D FISH analysis described here, we used the RT class-specific genome annotations to design three pools (Early-S, Middle-S, and Late-S) of oligo-painting FISH probes along the short arm of chromosome 5. These RT-specific painting FISH probes were used to stain genomic regions in formaldehyde-fixed nuclei. Preliminary quantitative 3D FISH analyses indicated that Early-S FISH paints preferentially colocalized with weak DAPI signals, relative to adjacent same-nucleus euchromatin control regions. Similarly, Middle-S FISH paints are preferentially colocalized with strong DAPI signals. Together, these findings support the existence of the two spatially distinct compartments in maize interphase euchromatin. If further validated by HiC analysis, the mini-domain model may serve as a new conceptual framework for understanding chromatin structure-function relationships in plants.

Funding acknowledgement: National Science Foundation (NSF)

P110

Genomic conflict between the A and B chromosomes in High Loss lines

(submitted by James Birchler <birchlerj@missouri.edu>)
Full Author List: Albert, Patrice S.[1]; Yang, Hua[1]; Gao, Zhi[1]; DeVore, Cassidy[1]; Birchler, James A.[1]
[1] University of Missouri; Division of Biological Sciences; Columbia, Missouri, USA 65211

The B chromosome of maize is a nonvital chromosome but has a drive mechanism consisting of nondisjunction at the second pollen mitosis that produces the two sperm and then the sperm with the B chromosomes preferentially fertilizes the egg rather than the central cell. The so-called High Loss line discovered by Rhoades and Dempsey shows breakage of heterochromatic knobs on the A chromosomes at the second pollen mitosis when B chromosomes are present, which was suggested as a related phenomenon to the B nondisjunction. The High Loss line also shows the production of A chromosome trisomies and triploids when B chromosomes are present. Examination of mature pollen of this line with B chromosomes shows chromatin bridges between sperm and a high percentage (up to 20%) of grains with only a single diploid sperm, the latter explaining the production of triploids. Crosses to tetraploids and two day pollinations with phenotypic markers were conducted to gain insight into the latter effect on the A chromosomes and the consequences of the resulting fertilization events. Progeny tests of different classes of kernels from crosses to an *a1* tester were conducted to understand the two products of the breakage at the second pollen mitosis. The High Loss line was also tested for whether preferential fertilization occurred by crossing the High Loss line without B chromosomes by a *B-peru*-phenotypically-marked B chromosome. Fertilization was found to be the typical 2: 1 ratio in favor of the B chromosome-containing-sperm joining with the egg in the process of double fertilization. Thus, the genome wide impact of B chromosomes in the High Loss line does not affect the preferential fertilization aspect of genetic drive.

Gene / Gene Models described: *a1, B-peru*

Funding acknowledgement: National Science Foundation (NSF)

P111 @Citogenetica

Heterochromatic knobs exhibit differences in chromatin accessibility during maize development

(submitted by Mateus Mondin <mmondin@usp.br>)
Full Author List: Sattler, Mariana C.[1]; Bass, Hank W.[2]; Mondin, Mateus[1]
[1] ESALQ, University of São Paulo; Piracicaba, SP, Brazil, 13418-900
[2] Department of Biological Science, The Florida State University; Tallahassee, FL, USA

Maize knobs are composed of in tandem array repeats belonging to two different families, K10 and TR-1. Knobs have been recognized as homogenous and static structures, being classified as constitutive heterochromatin. Nevertheless, the advance in genomic analysis shows the knobs as a more complex structure in terms of organization and dynamics. A detailed analysis of the K180 repeat family carried out by our group evidenced two distinct subfamilies, named K180_1 and K180_2. Aiming to unravel the distribution and chromatin status of each subfamily, we annotated K180_1 and K180_2 to the reference genome of Z. mays B73v5 using the UCSC Genomaize tools (genomaize.org). Data distribution was intersected with DNA replication timing profiles and chromatin accessibility for four tissues: earshot, endosperm, coleoptilar node, and root tip. The K180 repeats occur in long blocks coinciding with the cytological knobs (K5L, K6S, K7L, K8L, and K9S) and are dispersed in small clusters along the chromosomes. At the same time, the K180_2 subfamily occurred only as distributed. All five cytological knobs, composed majority of K180, exhibit a clear late replicating chromatin profile, as previously reported. However, the analysis of chromatin accessibility revealed a tissue-specific variation pattern to K180 cytological knobs. Among the four tissues, the endosperm exhibited the lowest level of chromatin accessibility in all five K180 cytological heterochromatin, followed by the coleoptilar node and the earshot. The root tip presented the highest levels of chromatin accessibility. Such tissue-specific variation pattern was not detected in the dispersed K180 repeat regions, nor in the cytological knobs composed by TR-1 repeats observed on chromosomes 4 and 6. These results evidence that knobs might be responsive structures instead only static heterochromatic blocks.

P112

Kinesin-10-like is a candidate adaptor protein that mediates the interaction between *KINDR* and knob180 repeats, facilitating meiotic drive of abnormal chromosome 10

(submitted by Mingyu Wang <mw36149@uga.edu>)
Full Author List: Wang, Mingyu[1]; Swentowsky, Kyle W[2]; Gent, Jonathan I[2]; Brady, Meghan J[3]; Dawe, R. Kelly[2 3]
[1] Institute of Bioinformatics, University of Georgia, Athens, GA 30602
[2] Dept of Plant Biology, University of Georgia, Athens, GA 30602
[3] Dept of Genetics, University of Georgia, Athens, GA 30606

Maize abnormal chromosome 10 (Ab10) encodes a meiotic drive system that converts two types of heterochromatic knobs into neocentromeres that are preferentially transmitted to progeny. In recent years, two neocentromere-activating genes encoding kinesins were discovered on Ab10, Kinesin driver (*Kindr*) and *TR-1 kinesin* (*Trkin*) that activate knob180 knobs and TR-1 knobs individually. However, it is unclear how either kinesin physically associates with knobs. We have discovered a mutant of Ab10 called *smd13* in which *KINDR* is expressed normally but does not show meiotic drive. Immunolocalization data suggest that this mutant lacks a gene required for *KINDR* association with knob180 knobs. Using de novo transcriptome assembly, we identified a gene with homology to *Kinesin-10* that has lower expression in the *smd13* mutant relative to wildtype Ab10. It is encoded in six tandem copies immediately adjacent to the ten tandem copies of Kindr on the distal tip of Ab10. Three of the copies are deleted in the *smd13* mutant. Here we will describe our data on the structure and function of the candidate *Kinesin-10-like gene* and speculate on its role in Ab10 meiotic drive.

Funding acknowledgement: National Science Foundation (NSF)

P113

Stability of chromosome segregation by synthetic maize centromeres

(submitted by Yibing Zeng <yz77862@uga.edu>)
Full Author List: Zeng, Yibing[1]; Dawe, R. Kelly[1]
[1] Department of Genetics; University of Georgia; Athens, GA, USA 30602

Creation of synthetic chromosomes has the potential to allow for large-scale manipulation of genetic pathways in vivo. A key step in the formation of synthetic chromosomes is the synthesis of functional centromeres. In plants, centromeres are not defined by DNA sequence but by a histone variant, Centromeric Histone H3 (CENH3). CENH3 has a crucial role in specifying centromere position and loading the machinery necessary to segregate chromosomes. In previous work, we showed that Arrayed Binding Sites (ABS) containing LexO arrays inserted into chromosome 4 long arm (4L) could recruit the DNA binding protein LexA fused with CENH3 (LexA-CENH3). LexA-CENH3 bound to ABS arrays and recruited native CENH3. Activation of centromeres at ABS loci induced chromosomal breakage, releasing fragments of chromosome 4L and at the same time rescuing transmission of the fragments with newly formed ABS centromeres. So far, we have identified four neochromosomes (Neo4Ls), each of which has been transmitted through meiosis for at least two generations without LexA-CENH3, indicating the formation of functional neocentromeres. Nevertheless, the new centromeres can be unstable during plant growth, gametogenesis, or fertilization. The instability of the new centromeres can be explained by altered genome dosage, lack of telomeres, centromere-mediated chromosome erasure, or meiosis errors of single-copy chromosomes that lack pairing partners.

Funding acknowledgement: National Science Foundation (NSF)

P114 @aimeejschulz

A Seed Dispersal Game: curriculum for teaching plant domestication and adaptation to students of all ages

(submitted by Aimee Schulz <ajs692@cornell.edu>)
Full Author List: Schulz, Aimee J[1]; Khaipho-Burch, Merritt[1]
[1] Section of Plant Breeding and Genetics, Cornell University, Ithaca, NY USA 14853

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.

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

P115
DNA extraction method from a seed without damaging to germination ability in maize (*Zea mays* L.)

(submitted by Gibum Yi <gibumyi@gmail.com>)
Full Author List: Kim, Jae-Hong[1]; Kim, Ji Won[1]; Yi, Gibum[1]
[1] Department of Bio-Environmental Chemistry, College of Agriculture and Life Sciences, Chungnam National University, Daejoen, 34134, Republic of Korea

In order to preserve excellent genetic resources and exploit them to breed better cultivars for food security, various studies on maize are underway. Maize also provide great materials for genetic studies accompanying with genotyping and phenotyping at the same time. DNA extraction is essential processes for those studies. Extracting DNA from young leaves in seedling stage is advantageous because it will cause less damage to remaining plant which can be further used for phenotypic analysis. Seed DNA extraction is even more advantageous in terms of saving time, labor, space, and cost for germination. Therefore, we presented seed DNA extraction method which does not cause damage to the seed germination ability. DNA was extracted by CTAB method and commercial DNA extraction kit from the seed fragment and quantity and quality of the DNA was examined. Seed germination was tested for proportional seed cut in 0, 10, 30, and 50% of the proximal part of a seed proportionally by weight. When DNA was extracted from the upper seed fragments, high-quality and enough amount of DNA was obtained. Germination rate was not reduced in the range of 10-30% of seed cut by weight. These results indicate that DNA extraction method from seeds can be an efficient way for the samples for genotyping and phenotyping, and this method can be also applied to high throughput DNA extraction in maize and possibly to other smaller seeds.

Funding acknowledgement: National Research Foundation of Korea

P116
Documenting successful applications of plant genetic resources

(submitted by Stephen Gray <sjgray4@iastate.edu>)
Full Author List: Gray, Stephen J.[1]; Mahama, Anthony A.[1]
[1] Iowa State University Department of Agronomy, Ames, Iowa, 50010

Plant genetic resources serve as a valuable tool for plant breeders as they enable access to a wide range of genetic diversity. Such genetic diversity can be harnessed to integrate traits necessary to meet breeding objectives. As a result, the exploitation of plant genetic resources has led to significant increase in cultivar productivity. Successful conservation and application of plant genetic resources in the future in part relies on providing the tools necessary for individuals to advance their knowledge on the subject. Therefore, a focus has been placed on developing educational materials on plant genetic resource conservation and use. A component of the educational material developed is a success stories eBook which documents the successful application of plant genetic resources. The success stories eBook provides the reader with a description of the problems addressed by a particular breeding program and breaks down how plant genetic resources were used to achieve breeding objectives. The development of the open-source success stories eBook will provide the public with a freely and easily accessible resource which can be used to advance the understanding of plant genetic resource use and its impact.

Funding acknowledgement: United States Department of Agriculture (USDA)

P117

Effects of plot orientation and leaf angle on maize grain yield

(submitted by Gregory Schoenbaum <gregorys@iastate.edu>)
Full Author List: Schoenbaum, Gregory R.[1]; Alladassi, Boris M.E.[1]; Li, Xianran[2]; Yu, Jianming[1]
[1] Department of Agronomy, Iowa State University, Ames, IA, USA 50011
[2] USDA-ARS, Wheat Health, Genetics, and Quality Research, Pullman, WA, USA 99164

Maize canopy architecture is an important determinant of light interception, which has a profound effect on maize grain yield. Plant breeders have utilized this knowledge to produce hybrid maize varieties with canopies that attempt to maximize light interception at high population densities. However, excessively high densities can have detrimental effects on maize, including barrenness and increased lodging, as well as to the producer via expensive equipment modifications and higher seed and fertilizer costs. The aim of this research is to investigate whether a combination of plot orientation and canopy architecture exists that will increase grain yield with minimal or no increases in population density. This experiment will consist of two replications for each maize hybrid entry in a randomized complete block design performed at two locations in Iowa, USA over three seasons. Twenty maize test cross hybrids with various canopy types (flat, erect, or dynamic) will be planted in two-row plots with orientations either parallel or perpendicular to the path of incoming solar radiation. Measurements taken on each plot will include canopy height and light penetration at various growth stages, flowering time, lodging, and yield. These data will then be analyzed to determine whether plot orientation influences light interception and yield and, if so, to what extent. Findings from this study may have future value in accurately predicting optimum planting densities based on canopy architecture and plot orientation. t;Smart Hyperlink"/>

Funding acknowledgement: National Science Foundation (NSF)

P118

Encouraging early pursuit of experiential learning in STEM education

(submitted by Brandi Sigmon <bsigmon2@unl.edu>)
Full Author List: Sigmon, Brandi[1]
[1] Department of Plant Pathology; University of Nebraska-Lincoln; Lincoln, NE, USA 68583

Student participation in experiential learning is essential to gain valuable hands-on experience in preparation for their future careers. However, many STEM students wait too long before participating in experiential learning, which can result in missed opportunities impacting academic success, retention, and career preparedness. Early programming to address knowledge gaps and student confidence may encourage earlier participation in experiential learning. In this study a group of 22 first- and second-year undergraduate students enrolled in an introduction to the major STEM course received targeted instruction surrounding topics relating to experiential learning and participated in an experiential learning-oriented workshop and research symposium. Both quantitative and qualitative data were collected and analyzed from assessments administered throughout the semester to determine the impact of these activities on student learning. Students were found to be motivated to pursue undergraduate research and understood the value of gaining this experience but less confident in how to obtain these opportunities. Students were also less confident in their knowledge of other types of experiential learning outside of undergraduate research. Through targeted instruction and activities including participation in an experiential learning-oriented workshop and research symposium, student confidence in their knowledge of obtaining experiential learning opportunities increased significantly. Students also broadened their interests and planned to participate in more diverse experiences while maintaining their plans to pursue undergraduate research experiences. Early programming addressing knowledge gaps and student confidence in introductory STEM courses may therefore empower students to start seeking opportunities earlier in their undergraduate education.

P119

Abstract has been removed from the program.

P120

Managing and distributing maize diversity: The NCRPIS maize collection

(submitted by Vivian Bernau <vivian.bernau@usda.gov>)
Full Author List: Bernau, Vivian[1]; Millard, Mark[1]; Peters, David[1]
[1] USDA/ARS Plant Introduction Research Unit and Iowa State University Department of Agronomy, Ames, Iowa

The USDA National Plant Germplasm System includes a collection of more than 20,000 accessions of cultivated temperate- and tropical-adapted maize and wild relatives from around the world. This collection is held at the North Central Regional Plant Introduction Station (NCRPIS) in Ames, Iowa. Currently, approximately 75% of the collection (15,347 accessions) is available for distribution upon request. Seed viability of each accession is monitored on 10-15 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 359,932 trait observations on 17,201 accessions, and 42,969 ear, kernel, and cob images on 16,624 accessions. Germplasm requests can be made through the NPGS GRIN-Global public website. In 2022, more than 19,000 packets of maize were distributed by NCRPIS to requestors across the country and around the world.

P121

The Maize Building Better Together Workshop: Strategies for a healthy and inclusive research community

(submitted by Irina Makarevitch <imakarevitch01@hamline.edu>)
Full Author List: The MGC Committee on Outreach, Diversity, Inclusion, and Education (CODIE)[1]
[1] https://www.maizegdb.org/mgc/outreach/index.php

The COVID-19 pandemic highlighted underlying pervasive systemic vulnerabilities in our scientific communities, including the marginalization of historically-excluded groups, assaults on mental health within our research networks, and disproportionate stresses and job insecurities for early-career/junior scientists. In response, the Maize Genetics Cooperation's Committee on Outreach, Diversity, Inclusion, and Education (CODIE) sponsored a virtual workshop in January 2022 to help envision a more equitable and supportive future by engaging all members of our community. The workshop was designed to equip participants with tools to address immediate needs and develop strategies to promote widespread engagement and systemic change. The workshop panels covered navigating issues and impacts related to mental health in the workplace, building positive partnerships among diverse peers, growing inclusive scientific communities, and advancing inclusive environments through allyship. Invited panelists, plant scientists with relevant lived experience as well as experts in the areas of mental health, equity, and inclusion, led the discussions on these topics. Over 100 participants from the community engaged in two days of conversations. Community input was collected through the surveys and breakout room discussion notes. The pre-workshop survey indicated that 60% of participants had an increase in professional workload during the pandemic, underscoring the importance of discussing mental health and well-being in academia. Following the workshop, participants felt a greater level of comfort discussing mental health and well-being,] and felt more well-equipped to dismantle barriers to equity and inclusion to support colleagues of marginalized identities. In the post-workshop survey, participants emphasized the importance of fostering a culture that centers well-being, care, and empathy over productivity to maintain a healthy scientific community. CODIE will continue to work on developing strategies and promoting widespread engagement and systemic change through community conversations.

Funding acknowledgement: National Science Foundation (NSF)

P122 @hwb333

The *Maize-10-Maze* project, an educational public chromosome map garden featuring the mutants of maize.

(submitted by Bianca Sheridan <bsheridan@fsu.edu>)
Full Author List: Sheridan, Bianca K. M.[1]; Beechy, Yoel F.[1 2]; Flores, Tammy V.[1]; Hambuechen, Callie A.[1]; Jadoo, Dylan[1]; Davis, Kimberly[3]; Doster, Jonathan[1]; Hanley-Bowdoin, Linda K.[4]; Bass, Hank W.[1]
[1] Department of Biological Science, Florida State University, Tallahassee, FL, USA, 32306
[2] Honors/Gifted Externship, Leon High School, Tallahassee, FL, USA, 32308
[3] Florida A & M University, Tallahassee, FL, USA, 32307
[4] Plant and Microbial Biology, North Carolina State University, Raleigh, NC, USA, 27606

The ***Maize-10-Maze*** project is a public outreach event that utilizes a maize chromosome map garden to educate and engage the public on the importance of plant biology research and genetics. The event, next scheduled for Summer 2023 at Florida State University, features a self-guided tour of the maize genome organized along the karyotype of maize as part of our outreach project (NSF IOS # 2025811). Each of the 10 rows represents a single chromosome and the mutant families are in **chromological order**. The garden includes mutants that showcase visually striking plant or seed phenotypes, have agronomic importance, or have major scientific importance. The chromosome map garden in the *Maize-10-Maze* project showcases mutants that possess different characteristics, such as visually striking or unique plant or seed phenotypes like *Knotted1* or *lazy plant1*, agronomic importance like *brittle endosperm1*, and major scientific importance like *teosinte branched1*. High school and undergraduate college students developed and updated the weatherproof field placards that describe each mutant for self-guided public tours of the maize genome, introducing genetic concepts such as phenotypes, genotypes, modes of inheritance, genetic locations, and the status of molecular cloning. The event is co-sponsored with the Florida A&M University's Forestry and Conservation Education (**FACE**) Summer Program and aims to increase accessibility and longevity of the project through photography and online resources, including the @scienceforyall tiktok page and a website, crazylazycorn.org. Collectively, these super cool and exciting educational tools offer a fun, engaging, and interactive educational experience for a wide range of audiences including middle schools, universities, and the general public. The online tools will provide long lasting information for use in classroom instruction, virtual field trips, and other educational activities in perpetuity.

Funding acknowledgement: National Science Foundation (NSF), Varina Vaughn - Winona Jordan Scholarship, Biological Science Alumni Scholarship

P123 @vbern

The Wilkes Legacy Collection: The modern era of teosinte research began here

(submitted by Vivian Bernau <vivian.bernau@usda.gov>)
Full Author List: Bernau, Vivian[1]; Costich, Denise E.[2]; Wilkes, H. Garrison[3]
[1] North Central Region Plant Introduction Station (NCRPIS), Iowa State University Department of Agronomy and USDA-Agriculture Research Service, Ames, Iowa 50014
[2] Institute for Genomic Diversity, Cornell University, Ithaca, NY 14853
[3] Biology Department, University of Massachusetts, Boston, Massachusetts 02125 (Retired)

Garrison Wilkes's 1967 pre-molecular biology doctoral thesis set the stage for many advances in understanding the key players in maize evolution, especially the teosintes (all *Zea* taxa, excluding maize). His field work in the 1960s addressed the essential unknowns of the time: How many taxa are there? Where do they grow? How are they related to one another? How large are the populations? Are they freely hybridizing with maize? In the 60 years that have passed, Garrison has been a direct eyewitness of the erosion of these populations and has been a tireless advocate for their conservation. In 2022, Garrison donated his original collections of teosinte seeds to the USDA-ARS North Central Region Plant Introduction Station in Ames, Iowa. After decades of storage, the seeds are not viable. However, we have carefully captured images of the seeds, transcribed the information listed on each packet, and georeferenced the collection points of the 1600 samples. With this curated dataset—and limited seed available for sequencing—Garrison's pioneering work will continue to serve as the foundation of future scientific advances in the study of crop evolution, as well as the *in situ* conservation of the genetic diversity of the genus *Zea*.

P124

A LD decay method for determining confidence intervals of QTL in GWAS and perform QTL clustering

(submitted by Aurelien Beugnot <aurelien.beugnot@gmail.com>)
Full Author List: Beugnot, Aurélien[1]; Mary-Huard, Tristan[1 2]; Charcosset, Alain[1]; Moreau, Laurence[1]; Fievet, Julie[1]
[1] Université Paris-Saclay, INRAE, CNRS, AgroParisTech, GQE-Le Moulon, 91272 Gif-sur-Yvette, France
[2] MIA, INRAE, AgroParisTech, Université Paris-Saclay, 75005, Paris, France

In the last few years, the study of the genetic architecture of complex traits through the detection of Quantitative Trait Loci (QTLs) in genome-wide association studies (GWAS) has been facilitated by the increased accessibility of high-throughput genotyping data. In parallel, the growing interest in understanding adaptation to environmental conditions has increased the number of phenotyping data available (location, year, stress, traits). The QTL detection in one environment or one population is nowadays relatively easy, but the integration of all these individual QTL detection results (for several populations, multiple environments and different traits) to identify the position of QTLs that underlie the trait remains a tedious task. In this work, we proposed an original method to determine the confidence interval around each QTL, followed by a QTL clustering. This new method, based on the local LD decay (Hill and Weir regression), is applied a posteriori to the QTL detection. Our method used an iterative process accounting for local complex LD patterns due to population structure and recombination hotspots. Moreover, compared to other approaches based on an a priori physical or genetic distances to define confidence intervals, we provide a more accurate estimation of the QTL confidence intervals, particularly in the centromeric regions and the presence of long-distance LD. We applied this method on two hybrid panels with different levels of LD.

Funding acknowledgement: Institut National de Recherche pour l'Agriculture, l'Alimentation et l'Environnement (INRAE)

P125

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

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

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. We present a comprehensive multi-year analysis of changes to root system architecture that result from altered nodal root development, including 3D phenotyping of excavated root crowns. To explore differences in nutrient acquisition abilities, a nitrogen contrast field experiment was performed in 2022 comparing the Zmrt1-2 allele to the ZmRt1 wild type allele in high (150lb/ac) and low (50lb/ac) nitrogen conditions. Root crowns and 1m deep soil cores were taken to determine root distributions and root length density across the depth profile. 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.

P126 @SylvieCoursol

A combination of quantitative trait locus mapping and transcriptome analysis reveals a cluster of three MYC2- and jasmonic acid-dependent genes associated with chilling tolerance in maize

(submitted by Sylvie Coursol <sylvie.coursol@inrae.fr>)
Full Author List: Duran Garzon, Catalina[1]; Chemin, Claire[2]; Rau, Andrea[2 3]; Decaux, Benoît[4]; Sellier-Richard, Hélène[2 4]; Drouaud, Jan[2]; Soubigou-Taconnat, Ludivine[5 6]; Brunaud, Véronique[5 6]; Martin, Marie-Laure[5 6 7]; Palaffre, Carine[8]; Domon, Jean-Marc[1]; Rayon, Catherine[1]; Lourgant, Kristelle[2]; Guénin, Stéphanie[9]; Gutierrez, Laurent[9]; Charcosset, Alain[10]; Coursol, Sylvie[11]; Giauffret, Catherine[2 12]
[1] Université de Picardie Jules Verne, Université de Liège, Université de Lille, INRAE, BioEcoAgro, Biologie des Plantes et Innovation, 80039, Amiens, France
[2] INRAE, Université de Liège, Université de Lille, Université de Picardie Jules Verne, BioEcoAgro, AgroImpact, 80203, Péronne, France
[3] Université Paris-Saclay, INRAE, AgroParisTech, GABI, 78350 Jouy-en-Josas, France
[4] INRAE, Unité Expérimentale Grandes Cultures Innovation et Environnement, 80203, Péronne, France
[5] Université Paris-Saclay, CNRS, INRAE, Université Evry, Institute of Plant Sciences Paris-Saclay (IPS2), 91190, Gif-sur-Yvette, France
[6] Université de Paris Cité, Institute of Plant Sciences Paris-Saclay (IPS2), 91190, Gif-sur-Yvette, France
[7] Université Paris-Saclay, INRAE, AgroParisTech, UMR MIA Paris-Saclay, 91120, Palaiseau, France
[8] INRAE, Unité expérimentale du maïs, 40390, Saint-Martin-de-Hinx, France
[9] Université de Picardie Jules Verne, Centre de Ressources régionales en Biologie Moléculaire, 80039, Amiens, France
[10] Université Paris-Saclay, INRAE, CNRS, AgroParisTech, GQE-Le Moulon, 91190, Gif-sur-Yvette, France
[11] Université Paris-Saclay, INRAE, AgroParisTech, Institut Jean-Pierre Bourgin (IJPB), 78000, Versailles, France
[12] Present address: INRAE, CIRAD, Université de Montpellier, Institut Agro Montpellier, AGAP Institut, 34398, Montpellier, France

Due to climate change, conditions are less favourable for spring crops in the summer. Earlier sowing dates are part of a remedial strategy that requires improved crop chilling tolerance. Although modern maize hybrids have been greatly improved for chilling tolerance, examination of important maize progenitors shows that defective alleles are hidden in current breeding populations. Here, we combined quantitative genetics and transcriptome analysis to elucidate the genetic architecture of chilling tolerance in European maize. Linkage analysis with a doubled haploid (DH) population derived from two European dent inbred lines revealed two hotspots of quantitative trait loci (QTL) with potentially pleiotropic effects on chilling tolerance. These two QTL hotspots were further analysed by transcriptome profiling under control and chilling conditions of two closely related but phenotypically distinct sister lines from the DH population. Using mRNA-seq data from leaves, we identified 574 genes showing significant variation in gene expression attributable to genetic variation. Validation using quantitative RT-PCR and comparison with QTL allelic effects reduced the set of positional candidates to three regulated target genes of the two transcription factors ZmMYC2s that exert a central function in jasmonic acid signaling. Overall, our results open the possibility of improving rapid temperature adaptation in maize by co-selection of favourable alleles.

Gene / Gene Models described: *ZmMYC2s*; Zm00001d030028, Zm00001d047017
Funding acknowledgement: French National Agency for Research (ANR), German Federal Ministry of Education and Research (BMBF), Spanish Ministry of Science and Innovation (MICINN), France Agrimer, Conseil Régional Hauts de France, FEDER

P127

A comprehensive phenomics study of above and below-ground traits in a maize diversity panel under nitrogen limitation

(submitted by Sumeet Mankar <sumeetmankar171@gmail.com>)
Full Author List: Mankar, Sumeet[1]; Luebbert, Collin[1]; Rajukar, Ashish[2]; Leakey, Andrew[2]; Baxter, Ivan[1]; Topp, Christopher[1]
[1] Donald Danforth Plant Science Center, Saint Louis, MO 63132, USA
[2] Department of Plant Biology, Institute for Genomic Biology, University of Illinois, Urbana, IL 61801, USA.

Reductions of synthetic nitrogen fertilizer use in industrial maize production is a key element in developing climate-smart food production systems with smaller greenhouse gas footprints. Improved efficiency of maize root systems offers a plausible and underexplored solution to balance the need for more sustainable agriculture while meeting global food demands. While recent studies have begun to identify genes that influence root system architecture, their relationships to efficient nitrogen capture and whole plant physiological and agronomic traits are largely unknown. We undertook a large-scale, comprehensive evaluation of root and shoot phenotypes (261 traits total) in low nitrogen fields across 2 years in a subset (272 inbred lines) of the Buckler-Goodman diversity panel. Genome-wide association analysis of minirhizotron datasets and 188 traits for 2D and 3D root crown phenotypes revealed 55 marker-trait associations (MTAs) for root system architecture and root length density up to 1.4 meters. As a measure of root system function, ~4000 plants were sampled for leaf and kernel ionomics, yielding 649 MTAs. We collected yield, plant N, and X other aboveground traits which identified a further 1255 MTAs. We identified several QTL overlaps across years and genotypes. This study provides a foundation for the genetic dissection of complex whole plant traits that may contribute to nitrogen uptake efficiency and more productive, more sustainable maize agriculture. **Keywords**: Nested-Association mapping, nitrogen use efficiency, root phenomics

P128

A leucine rich repeat receptor kinase gene confers quantitative susceptibility to maize southern leaf blight

(submitted by Peter Balint-Kurti <pjbalint@ncsu.edu>)
Full Author List: Chen, Chaun[1]; Zhao, Yaqi[1]; Tabor, Girma[2]; Nian, Huiqin[1]; Phillips, Joanie[2]; Wolters, Petra[2]; Yang, Qin[1 3]; Balint-Kurti, Peter[3 4]
[1] State Key Laboratory of Crop Stress Biology for Arid Areas and College of Agronomy, Northwest A&F University, Yangling 712100, China
[2] Corteva Agriscience, Johnston, IA , USA
[3] Dept of Entomology and Plant Pathology, North Carolina State University, Raleigh 27695, NC, USA.
[4] Plant Science Research Unit, USDA-ARS, Raleigh 27695, NC, USA

Southern leaf blight (SLB), caused by the necrotrophic fungal pathogen *Cochliobolus heterostrophus* (anamorph Bipolaris maydis), is a major foliar disease which causes significant yield losses in maize worldwide. A major quantitative trait locus, qSLB3.04, conferring recessive resistance to SLB was previously mapped on maize chromosome 3. Using a combination of map-based cloning, association analysis, ethyl methanesulfonate (EMS) and transposon mutagenesis and CRISPR-Cas9 editing, we demonstrate that a leucine-rich repeat receptor-like kinase gene which we have called ChSK1 (Cochliobolus heterostrophus Susceptibility Kinase 1) at qSLB3.04 causes increased susceptibility to SLB. Genes of this type have generally been associated with the defense response. We present evidence that ChSK1 may be associated with suppression of the basal immune response. These findings contribute to our understanding of plant disease susceptibility genes and the potential to use them for engineering durable disease resistance.

Gene / Gene Models described: *ChSK1*; Zm00001d039822

Funding acknowledgement: National Science Foundation (NSF), United States Department of Agriculture (USDA), National Natural Science Foundation of China, National Key Research and Development Program of China

P129 @Cornontherocks

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

(submitted by Zachary Traylor <zbtyxb@umsystem.edu>)
Full Author List: Traylor, Zachary B.[1]; Hall, Garret[2]; Hinegardner, Gary[3]; Somavat, Pavel[4]; Braun, David M.[1]; Flint-Garcia, Sherry[5]
[1] Division of Biological Sciences, University of Missouri; Columbia, MO 65211
[2] Division of Plant Science and Technology, University of Missouri; Columbia, MO 65211
[3] Wood Hat Spirits; New Florence, MO 63363
[4] Division of Food Science, University of Missouri; Columbia, MO 65211
[5] USDA-ARS Plant Genetics Research Unit; Columbia, MO 65211

With the growing interest in exploring the genetic diversity of maize, there has been a wave of new genomic and biochemical methods to dissect the differences between previously understudied heirlooms and better understand diversity at a molecular level. Along with this focus in diversity, traditional association and QTL methods as well as a range of biochemical assays are regularly used to study agronomic traits and characteristics relevant to maize productivity but have been less focused on food applications to date. There is likely a plethora of metabolites that contribute to the flavor profiles of these maize lineages which require more in-depth, targeted analyses that we just recently initiated. Currently the methods aimed at investigating nuances in metabolite identity and quantity are low-throughput and involve high investments in the amount of seed needed for study as well as the cost to process these seed into food and beverage products. It is here that we have been able to fill this gap by designing a method to utilize minimal amounts of seed to process kernels into a fermented beer and alcohol distillate that resembles the mainstream whiskey production schemes used in the US market. Our goal is to process a single ear of corn into distillate that is sufficient to study metabolite profiles during the fermentation process as well as those present after the distillation process to study variance across corn germplasm. This platform will allow for future applications of metabolite characterization and quantification and will lead into larger studies of the genetic control of these metabolites and a way to track and select for metabolite composition in maize.

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

P130

A novel multi-objective genomic selection strategy improves long-term genetic gains through improved diversity maintenance

(submitted by Robert Shrote <shrotero@msu.edu>)
Full Author List: Shrote, Robert Z.[1]; Thompson, Addie M.[1]
[1] Michigan State University; 1066 Bogue Street; East Lansing, Michigan, USA 48823

In recent years, the importance of maintaining genetic diversity for long-term genetic gain in a genomic selection program has become apparent. Several strategies have been proposed to combat the loss of valuable genetic diversity including optimal contribution selection (OCS) and its variants, weighted genomic selection (WGS), optimal haploid value selection (OHV), and optimal population value selection (OPV). All aforementioned selection techniques are treated as constrained, single-objective optimization problems. Here, we propose a novel, multi-objective genomic selection (MOGS) technique which accounts for two competing objectives: one inspired by conventional genomic selection (CGS), the other inspired by OPV. By mapping the Pareto frontier representing best allocation trade-offs for both objectives and selecting a single point according to user preference, we are able to create a selection technique capable of providing good long-term genetic gains while maintaining intermediate levels of genetic diversity. MOGS outperforms its progenitors CGS and OPV in several metrics, but underperforms in many metrics when compared to WGS, suggesting that improvements can be made to this multi-objective selection technique.

Funding acknowledgement: United States Department of Agriculture (USDA), NSF NRT IMPACTS Program, MSU Plant Science Fellowship, MSU Plant Resilience Institute

P131

A practical and cost-effective method to improve the efficiency of seed production for genetics research and breeding operations

(submitted by Dylan Schoemaker <schoemaker@wisc.edu>)
Full Author List: Schoemaker, Dylan L.[1]; McFarland, Frank[1]; Martinell, Brian[2]; Michel, Kathryn J.[1]; O'Brien, Dan[3]; de Leon, Natalia[1]; Kaeppler, Shawn M.[1 2]
[1] Department of Agronomy, University of Wisconsin – Madison, 1575 Linden Drive, Madison, WI 53706
[2] Wisconsin Crop Innovation Center, University of Wisconsin – Madison, 8520 University Green, Middleton WI 53562
[3] O'Brien Hybrids – 552 Glenway Road, Brooklyn, WI 53521

Manual pollination is a critical part of genetics and breeding research to create desired research crosses and to increase inbred and hybrid seed. Generally, maize pollinations are made by placing a paper pollinating bag on the tassel 24 hours prior to pollination ensuring that receptive silks of target plants are pollinated with newly released pollen. Pollination success in the field and in controlled environments is dependent upon sufficient high-quality pollen available at the time that recipient silks are receptive. However, maize pollen is short lived due to rapid water loss across time in field conditions. We have developed a quick and cost-effective method to gather pollen and mix it with a substrate to facilitate utilization of stored maize pollen for seed production. The method has been empirically evaluated using a seed production nursery in Verona, WI over three summers and across multiple planting dates. Multiple substrates were tested, and polyethylene (PEM) and polyetheretherketone (PEEK) based substrates were found to effectively extend the viability of stored maize pollen. PEEK-MP140 manufactured from PolyClean Technologies Inc. was the most effective at approximately $0.07 per gram compared to $15.00 per gram for PEM, therefore was selected for advanced field evaluation. Experimental results in 2022 demonstrated that mixed pollen kept at 6.1°C can effectively produce kernels after five days of storage and resulted in some success after nine days of storage. Mixed pollen stored up to 48 hours was shown to be effective when evaluated using pollen from 24 inbred lines and was usually equally effective and sometimes outperformed the standard manual pollination process. The proposed method improves the efficiency of breeding operations by increasing the number of pollinations possible per tassel and may aid in seed production for an array of genetic studies.

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

P132

A single-gene reduction in leaf area demonstrates potential for improved planting density tolerance in elite maize hybrids

(submitted by Matthew Runyon <mrunyon2@illinois.edu>)
Full Author List: Runyon, Matthew J.[1]; Sible, Connor N.[1]; Below, Fred E.[1]; Moose, Stephen P.[1]; Studer, Anthony J.[1]
[1] University of Illinois Urbana-Champaign; Urbana, IL, USA, 61801

Historical increases in planting density are a key driver of maize yield response under intensified management. Most modifications to plant and canopy architecture across elite maize germplasm facilitating improved planting density tolerance represent the cumulative effect of quantitative loci selected over time. A recessive, qualitative locus denoted as *reduced leaf area* (*rdla*) was previously characterized as conferring a 33% reduction in leaf area in an Oh43 inbred background. A 1Mb region on the long arm of Chromosome 4 containing this locus was introgressed into a panel of 28 expired Plant Variety Protection (ExPVP) inbred lines representing a diversity of germplasm sources, breeding eras, and native leaf areas. A subset of BC_2S_1 ExPVPs phenotyped in the 2021 field season demonstrated segregation consistent with single-gene action governing this trait and broadly showed reductions in leaf area consistent with what was observed in Oh43. Preliminary analysis of hybrids created from six BC_3S_1 and BC_4S_1 ExPVP introgression lines showed a 30-40% reduction in ear leaf area compared to hybrids created from their wild type counterparts in the 2022 field season. Under constant planting densities, yield and ear leaf area showed a strong positive correlation. However, reductions in grain yield from *rdla* were less severe than reductions in ear leaf area, demonstrating an improvement in the efficiency of grain produced per unit leaf area. Compositional analysis of grain and stover showed notable trends, suggesting differences in source-sink capacity and partitioning in *rdla* hybrids. Future experiments will evaluate *rdla* hybrids under elevated planting densities and across an expanded number of hybrid combinations. Altering leaf area by this magnitude via a single gene has strong potential to improve planting density tolerance in commercially relevant germplasm. Attempts to fine map the locus to a causative gene are currently being pursued.

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

P133

Adopting modern maize transformation technologies for sustainable crop improvement in Hawaii

(submitted by Jaclyn Nicole Uy <uyjnr69@hawaii.edu>)
Full Author List: Uy, Jaclyn Nicole[1]; Amore, Teresita[1]; Muszynski, Michael[1]
[1] Department of Tropical Plant and Soil Sciences, University of Hawaii, 3190 Maile Way, Honolulu,HI,96822

With increasing global temperatures, land and agriculture are being affected by factors such as variations in annual rainfall, fluctuations in temperatures, alterations in pest behavior, and rising sea levels. Maize, one of the most important seed and food crops in the world, will be severely impacted by climate change. Water deficits and temperature extremes can affect flowering and grain production. Accessing tropical diversity may be the key to increasing the adaptive potential of maize but breeding between temperate and tropical maize lines has been challenging due to crossing barriers. Tropical maize is photoperiod sensitive and will only flower and set seed under short-day conditions, unlike its temperate counterparts. The difference between photoperiod response hinders breeding between tropical and temperate lines. The University of Hawaii (UH) has a diverse collection of tropical maize germplasm that is a valuable source of climate adaptive traits. Our study aims to establish modern molecular tools at UH that will enable us to modulate photoperiod sensitivity in tropical maize. Recent advances in maize transformation methods such as ternary vector systems and morphogenic gene applications have tremendously improved precision breeding but have not been used on tropical lines. Here we use an auxotrophic *Agrobacterium* provided by Iowa State University, which harbors a ternary vector system for maize transformation. It has a binary vector (PKL2013) with *Cas9* and a sgRNA targeting the maize *glossy2* gene and a helper plasmid (pKL2299) that carries extra *Agrobacterium* virulence (*vir*) genes. We transformed immature embryos of the temperate inbred line B104, and the tropical maize inbred, Tzi8. We assessed the regenerative capabilities of Tzi8 (1- 11 plants per embryo) and B104 (1-10 plants per embryo) and produced *Cas9*-positive plants from both B104 (15) and Tzi8 (2). With this UH will be able to modulate photoperiod sensitivity in tropical maize lines.

Funding acknowledgement: National Science Foundation (NSF)

P134

Application of DH technology and molecular markers to combine multiple major genes for improving corn quality

(submitted by Tae-Chun Park <tcpark@iastate.edu>)
Full Author List: Park, Tae-Chun[1]; Kinney, Shelly[1]; Lauter, Adrienne M[2]; Scott, Paul M[2]; Aboobucker, Siddique I[1]; Frei, Ursula K[1]; Lübberstedt, Thomas[1]
[1] Department of Agronomy, Iowa State University, Ames, IA, 50011, USA
[2] USDA-ARS-CICGRU, Ames, IA, 50011, USA

The purpose of this study is to develop breeding methods for combining major genes or quantitative trait loci (QTLs) efficiently to improve the quality, value, and profit of varieties. For example, silage corn with the combination of several major genes is valuable for high digestibility and genetic purity conferred by the genes, *Opaque-2* (*o2*), *Brown midrib-2* (*bm2*), *Gametophyte factor-1* (*Ga1*), and Spontaneous haploid genome doubling (SHGD). Similarly, white waxy corn can be valuable for industrial purposes with *yellow 1* (*y1*), *waxy 1* (*wx1*), *Ga1,* and SHGD genes. Using doubled haploid (DH) technology, it is feasible to combine multiple major-effect genes in the haploid stage, which is difficult with traditional plant breeding. Four unlinked in the desired homozygous state would be expected to occur in one of 256 plants in diploid plants in a segregating F_2 population. As the number of target genes to be combined increases, the probability of having all desired alleles in a single individual is dramatically higher in the haploid phase than in the diploid phase. For example, the frequency of having the desired combination of five recessive alleles in the haploid stage, is one of the thirty-two. The minimal backcross population would be size 4,713 plants to have a 99% probability level of identifying an individual with five target genes in the correct state. By contrast, examination of only 145 DH plants, are required to identify an individual with five genes in the desired state at the 99% probability level. Using molecular markers allows efficient selection for desirable alleles in the haploid plants when phenotypes may not be visible. For these purposes, this study focuses on marker-assisted selection in haploid plants to efficiently transfer multiple major traits between genotypes in a breeding program.

Gene / Gene Models described: *o2, bm2, Ga1, SHGD, y1, wx1*; Zm00001eb378140
Funding acknowledgement: United States Department of Agriculture (USDA)

P135 @germanocneto

Association mapping for environmental-dependent alleles in maize is leveraged by climate and soil kinships

(submitted by Germano Costa-Neto <gmcn222@gmail.com>)
Full Author List: Costa-Neto, Germano[1]; Franco, Arcadio[1 2]; Hsu, Sheng-Kai[1]; Romay, M Cinta[1]; Hearne, Sarah[3]; Buckler, Edward[1 2 4]
[1] Institute for Genomic Diversity, Cornell University, Ithaca, NY 14853, USA
[2] School of Integrative Plant Sciences, Plant Breeding and Genetics Section, Cornell University, Ithaca, New York, 14853, USA
[3] International Maize and Wheat Improvement Center (CIMMYT), Texcoco, Mexico
[4] USDA-ARS, Robert W. Holley Center for Agriculture and Health, Ithaca, NY 14853, USA

In order to develop climate-resilient and resource-efficient varieties, breeding analytics could benefit from a cost-effective association of environmental-traits (ET) and genomics features, the so-called envGWAS. This approach explores the hidden effect of the historical environmental constraints that shaped the genetic makeup of local landraces or natural populations. Currently, envGWAS has the following limitations: (1) lack of high-quality public pipelines for pulling a wide number of ETs, (2) uncertainty on the power to detect true positive associations and; (3) existence of a confounding population structure due to the geographic proximity and shared ecological similarities (climate and soils). Here we develop a pipeline to collect a large number of ETs (n=155) and implement a flexible set of genomic/environmental covariates accounting for ecological relationship matrices. We used the maize Seeds of Discovery landrace data (n=2,910), considering ~720k SNPs, six envGWAS models, and three ETs as an example (cold, soil nitrogen, and organic carbon). We adopted two cutoffs for selecting significant SNPs, using -log10(p-values)>7.0, or selecting only the top 1% -log10(p-values). Gene Ontology (GO) enrichment was used to determine candidate genes' function for biological processes, cellular components, and molecular functions. Our results show that the inclusion of principal components from ecological kinship matrices could lead to a diverse set of results, yet complementary, from those achieved using genomic kinships. Consequently, it revealed different candidate loci aspects of the genetic makeup of the maize adaptation shaped by the envirome (core of environmental growing conditions). Despite the major part being model-specific, we observed GO terms overlapping across models and ETs, which could be useful to reduce false-positive associations. Ecological kinships for environmental-GWAS allow us to boost association mapping for environmental-dependent alleles. Hence, it could drive multi-objective solutions for breeding, which together could confer climate adaptation and resource efficiency use.

Funding acknowledgement: United States Department of Agriculture (USDA), Bill and Melinda Gates Foundation

P136

Breeding BeerCorn, maize hybrids with enhanced performance for brewing beer

(submitted by Steve Moose <smoose@illinois.edu>)
Full Author List: Moose, Steve[1]; Heller, Nicholas[2]; Riggs, Darin[3]; Riggs, Matt[3]
[1] University of Illinois at Urbana-Champaign, USA
[2] Illinois State University, Bloomington, Illinois, USA
[3] Riggs Brewing Company, Urbana, Illinois, USA

Beer may be brewed from a variety of cereal grains, but the oil concentration in maize (4-5%) is much higher than barley, wheat or rice (1-2%). Both the lipids that comprise oil, as well as yellow carotenoid pigments, can oxidize in the final product to cause "staling" or off-flavors, thus reducing the shelf life of beers brewed from maize. The Riggs Brewing Company, founded by two brothers with a desire to brew beers from grains grown on their family farm, developed a process to use #2 yellow dent corn grain in brewing lager-style beers, but the high kernel oil reduced foaming and accelerated staling. Their search for alternatives led to interest in Illinois Low Oil (ILO), a population derived from the open-pollinated variety "Burr's White" that contains only 1.5-2% oil due to long-term recurrent selection for low grain oil concentration. Initial results brewing with ILO were promising, but with low grain yields. To improve grain yield and other agronomic traits, ILO was hybridized to an inbred line derived from Illinois Low Protein, another population selected from Burr's White where low grain protein concentration is associated with higher starch. The ILO x ILP hybrids yield approximately 80 bushels per acre of white grain with 2.5% oil, which is now used to produce a popular local beer named American Lager. To further improve the agronomics of "BeerCorn", the ILP inbred has been crossed to white-seeded exPVP inbreds and progenies selected for reduced lodging, earlier maturity, higher grain yields, and less than 2.5% oil when combined with ILO. The ILO parent is also being improved by selecting for earlier maturity and reduced lodging. Our results illustrate yet another example of a valuable end use for the extreme seed compositions generated from the Illinois Long Term Selection experiment.

Funding acknowledgement: United States Department of Agriculture (USDA)

P137

Breeding for color and nutritional content in sweet corn and popcorn

(submitted by Jonathan Niyorukundo <niyorukundoj@gmail.com>)
Full Author List: Niyorukundo, Jonathan[1]; Wehrbein, Caleb[1]; Holding, David[1]
[1] University of Nebraska Lincoln; Lincoln, NE, 68588

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 opaque-2 (o2) mutation results in reduced alpha-zeins, and increased lysine-rich non zeins. 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 varieties known as Quality Protein Maize (QPM) were used as o2 donors and were crossed to multiple sugary-1 and shrunken-2 sweet corn and colored popcorn varieties. Colored QPP introgressions are progressing through selfing and backcrossing to recurrent popcorn parents for the necessary increase the popcorn background while selection of vitreous kernel (modified) lines carrying the gamma zein o2 modifier gene. QPP lines are currently advanced to the BC2 generation. After generating the BC3 generation, and several rounds of selfing, inbred lines will be selected for hybrid production. For QPS breeding, F2 kernels visibly segregating for both o2 and sweetcorn have been advanced through the F4, have been field selected for sweetness and texture and have been frozen for biochemical testing for sucrose, glucose and fructose, amino acids, total starch, resistant starch and microbiome characteristics. In addition to protein quality, we aim to improve sweet corn micronutrient content and aesthetic appeal by breeding for color diversity. Therefore, sweet corn lines were crossed to various colored dent corn varieties and have been similarly advanced through the F4 generation with several rounds of selection for color, sweetness and texture at 20 days after pollination (prime-eating stage). All the above biochemical tests on frozen sweet corn are in progress for these colored sweetcorn lines. Several standout QPS and colored sweetcorn lines have already been identified and we will present summaries of current progress in our popcorn and sweet corn breeding programs.

P138

Breeding of maize double recessive mutant lines using selection markers

(submitted by Young Sam Go <ysgo@korea.kr>)
Full Author List: Go, Young Sam[1]; Ha, Jun young[1]; Bae, Hwan Hee[1]; Son, Jae Han[1]; Kang, Kyeong Min[1]; Son, Beom Young[1]; Jung, Tae Wook[1]
[1] Department of Central Area Crop Science, National Institute of Crop Science, Rural Development Administration, Suwon, 16429, Republic of Korea

Waxy and sweet maize are popular vegetable food and important raw material for processing in republic of Korea. *wx* gene (waxy) changes the composition and structure of starch in endosperm and *se* gene (sweet) involves in endosperm storage starch. In order to make a breeding material and mutant lines constituting new starch in maize, F_1 seeds created by crossing *wx* and *se* mutant and bred by selfing in 2013. According to Mendel's genetic law, the genotype WxwxSese: wxwxSese : Wxwxsese : wxwxsese is separated as 9:3:3:1. We sowed F_2 seeds to select double mutant lines in field and crumpled seeds, a phenotype of sweet maize, were isolated in 2014. Generation progress was made over a period of 5 years (2015~2019) to fix the double mutant lines. And then, it was selected using reported functional markers related to *wx* and *se* genes in 2021. As a result of performing molecular marker analysis of 1000 double mutant lines, 37 lines were identified as double mutant lines. Growth characteristics, such as plant height, lodging, plant disease and pest were investigated in 37 lines. Among them, we finally selected 17 double mutant lines with excellent growth characteristics and created a new waxy-sweet maize F_1 hybrids by crossing in 2022. Total starch content was highest in waxy maize, but no significant difference was found in starch component in waxy, sweet and waxy-sweet maize. Based on these results, we will develop new maize cultivar using double mutant lines and provide new starch contents as food processing materials.

Funding acknowledgement: Rural Development Administration(RDA)

P139

Breeding transformable haploid inducers for elite line genome editing

(submitted by Timothy Kelliher <Tim.Kelliher@syngenta.com>)
Full Author List: Delzer, Brent[2]; Egger, Rachel[1]; Kelliher, Tim[1]
[1] Syngenta Seeds Development, Janesville, Wisconsin, USA
[2] Syngenta Seeds Research, Durham, North Carolina, USA

The introduction of 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 these techniques 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 variety in a single cross, obviating recurrent selection and linkage drag from trait introgression. For HI-Edit to operate at commercial scale, an efficient transformable HI must be developed, but HI lines are not typically transformable given their aberrant reproductive characteristics. Leveraging marker assisted selection for HI genes (*matl*, *dmp*, R1, and C1) and four generations of phenotyping, we developed 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 single QTL for Stiff Stalk transformability was identified. These results may facilitate the development of commercially scalable HI-Edit for maize and other crops.

P140

CERIS-JGRA analysis of flowering time in CML277 and Tzi8

(submitted by Joseph DeTemple <detemplj@iastate.edu>)
Full Author List: DeTemple, Joseph M[1]; Tibbs-Cortes, Laura E[1]; Wei, Jialu[1]; Li, Xianran[2]; Yu, Jianming[1]
[1] Agronomy Department, Iowa State University, 716 Farm House Ln, Ames, IA 50011
[2] USDA-ARS, Wheat Health, Genetics, and Quality Research, Pullman, WA 99164 USA

Genotype by Environment (GxE) effects are important for understanding plasticity and genotypic means in diverse genetic lines and different environments. The Maize Nested Association Mapping (NAM) families provide a GxE context within which individual traits, such as Flowering Time (FT), can be studied and compared. For this study, we looked at Recombinant Inbred Lines (RILs) from the CML277 and Tzi8 families, two tropical NAM founders with potential for genetic transformation, and compared the genotypic mean and plasticity of Flowering Time across environments. This comparison was done using the CERIS-JGRA approach, where environmental factors are replaced with an environmental index—the combination of a factor over a specified time window. By identifying temporal windows of high correlation with phenotypes, the selected environmental indices are relevant to early-season development as well as end-of-season phenotypes. QTL analysis was performed on the slope and intercept of these lines to find genetic loci associated with the identified environmental indices.

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

P141

Characterization and mapping of vascular and nonvascular colonization of maize *by Xanthomonas vasicola* pv. *vasculorum*

(submitted by Alex Mullens <mullens3@illinois.edu>)
Full Author List: Mullens, Alexander[1]; Jamann, Tiffany[1]
[1] University of Illinois; 1102 S. Goodwin Avenue; Urbana, IL 61801

Understanding how pathogens interact with different host tissue types is crucial to developing more robust disease resistant varieties. The vascular xylem and nonvascular parenchyma tissues represent distinct habitats within a plant for pathogenic bacteria to colonize. Each tissue type offers differing structural and physiological challenges for the pathogens to overcome to survive in each environment. Likewise 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)

P142

Combining heterotic groups for maize root seedling traits increases the power of genomic prediction accuracy under low phosphorus

(submitted by Alison Uberti <auberti@iastate.edu>)
Full Author List: Uberti, Alison[1 2]; Caixeta, Diego Gonçalves[2]; Bastos, Abel Jamir Ribeiro[2]; Schuster, Andreia[2]; Lübberstedt, Thomas[1]; DeLima, Rodrigo Oliveira[2]
[1] Department of Agronomy, Iowa State University, Ames, IA 50011, USA
[2] Department of Agronomy, Federal University of Viçosa, Viçosa, MG 36570, Brazil

The ability to predict superior genotypes with high accuracy is of key importance in maize breeding program, especially in root traits. Lateral root length plays an important role in the uptake of immobile nutrients such as phosphorus (P) by increasing soil exploration and P acquisition, mainly under low P. Here, we assess the potential of genomic prediction for maize root traits under contrasting phosphorus conditions. Lateral root length (LRL), root shoot ratio (RSR) and plant height (PH) were evaluated in a set of 148 tropical inbred lines in a greenhouse, under two P conditions: applied (AP) and non-applied P (NAP). The lines were genotyped with 3,083 SNPs markers and the prediction accuracy was estimate using reproducing kernel Hilbert spaces (RKHS) model. Genomic estimated breeding values (GEBVs) were calculated for eight scenarios. First, we estimated GEBVs by randomly attributing 30 and 60% of lines as training set. Then, the inbred lines were separated according to their three heterotic groups and we estimated GEBVs across all possible combinations of groups, i.e., one or two heterotic group predicting another. We observed that under both phosphorus conditions, AP and NAP, the GEBVs extracted by combining heterotic groups in the training set showed higher prediction accuracies than when only one heterotic group was used separately or when random sets of inbred lines were used to train the model for all tested root traits. When we used the combined heterotic groups I and II to train the model and predict group III, the prediction accuracies values were higher and ranged from 0.70 (RSR-NAP) to 0.75 (LRL-AP). In general, the predictions accuracies were higher in AP than NAP, however, the GEBVs changed considerably between P conditions. We concluded that the combination of heterotic groups improves the efficiency of genomic prediction of root seedling traits in maize under AP and NAP conditions.

Funding acknowledgement: CAPES, CNPq, FAPEMIG

P143

Comparison of genomic selection models for maize seedling traits under contrasting phosphorus conditions

(submitted by Alice Silva Santana <asantana@iastate.edu>)
Full Author List: Santana, Alice Silva[1 2]; Pereira, Gabriela dos Santos[2]; Filho, Vidomar Destro de Souza[2]; Miranda, Letícia Prada de[2]; Lübberstedt, Thomas[1]; DeLima, Rodrigo Oliveira[2]
[1] Department of Agronomy, Iowa State University, Ames, IA 50011, USA
[2] Department of Agronomy, Federal University of Viçosa, Viçosa, MG 36570, Brazil

Maize varieties with better root development have good nutrient acquisition and may have greater grain yield under limited phosphorus (P) condition than varieties with smaller roots. Genomic selection (GS) is a powerful tool that can be applied to select the best performing genotypes under P stress. Several GS models are available to account for genetic architecture of complex traits. Thus, we compared the ability of different GS models to predict plant height (PH), lateral root length (LRL) and root shoot ratio (RSR) in a maize panel of 148 tropical inbred lines from the breeding program of Federal University of Viçosa, Brazil. They were evaluated in a greenhouse, during 2016/2017 season, under two P conditions: applied (AP) and non-applied P (NAP). Prediction accuracies were compared using BayesA, BayesB, BayesC, Bayesian least absolute shrinkage and selection operator (Bayesian LASSO), and reproducing kernel Hilbert spaces (RKHS) models. The analysis was performed using 3,083 SNPs markers with 10-fold cross-validation and with a training population of 30 inbred lines. We observed that traits with higher values of broad-sense heritability were associated with higher prediction accuracy. Under AP, high prediction accuracies were obtained for PH (0.59-0.61), whereas moderate to low accuracies were observed for LRL (0.12-0.26) and RSR (0.20-0.27). RKHS outperformed all other models in predicting PH, LRL and RSR. Similar trend was observed under NAP condition. PH showed the highest prediction accuracies (0.31-0.49). The range of prediction accuracies across models were larger for LRL, ranging from 0.12 to 0.33, while RSR showed prediction accuracies ranging from 0.16 to 0.22. For PH and LRL, RKHS model showed the highest values for prediction accuracies. For RSR, the BayesB model showed the highest prediction accuracy. We concluded that RKHS and BayesB captured the linkage disequilibrium between markers and traits effectively leading to higher accuracies under contrasting P conditions.

Funding acknowledgement: CAPES, CNPq, FAPEMIG

P144

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

(submitted by Kiara Kappelmann <kiarak@iastate.edu>)
Full Author List: Kappelmann, Kiara[1]; Zhang, Bosen[2]; Scott, Marvin P.[1 3]; Edwards, Jode[1 3]; White, Wendy S.[4]; Li, Xianran[2 5]; Yu, Jianming[1]; Vanous, Adam[1 6]
[1] Department of Agronomy, Iowa State University, Ames, IA 50011, USA
[2] Department of Crop and Soil Sciences, Washington State University, Pullman, WA 99164 USA
[3] USDA-ARS Corn Insects and Crop Genetics Research Unit, USDA-ARS, Ames, IA 50011 USA
[4] Department of Food Science and Human Nutrition, Iowa State University, Ames, IA 50011 USA
[5] USDA-ARS Wheat Health, Genetics, and Quality Research Unit, USDA-ARS, Pullman, WA 99164 USA
[6] USDA-ARS North Central Regional Plant Introduction Station, USDA-ARS, Ames, IA 50014 USA

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 to uncover genetic pathways responsible for micronutrient concentration and forge a path using optimal training populations and model development for advancement through genomic-selection within GEM.

Funding acknowledgement: United States Department of Agriculture (USDA)

P145

Computational methods to identify ionome genes

(submitted by Lauren Whitt <lwhitt@danforthcenter.org>)
Full Author List: Whitt, Lauren[1 2]; Mahood, Elizabeth[1]; Barrett, Jennifer[1]; Ziegler, Greg[1];Dilkes, Brian[3];Baxter, Ivan[1]
[1] Donald Danforth Plant Science Center, Saint Louis, MO
[2] University of Missouri, Columbia, MO
[3] Purdue University, West Lafayette, IN

The ionome describes the concentration of elements acquired by plants and is heavily influenced by interactions between genes and the environment. Quantitative genetics and high-throughput pipelines have allowed for rapid identification of genomic markers associated with changes in the plant ionome, however, linking those markers to causal genes is more difficult. Many genes may be linked to one marker and annotations are ineffective for unknown causal genes, ultimately impeding candidate gene selection. To identify candidate genes influencing ionomic phenotypes, we assembled the known ionome gene (KIG) list: a curated collection of genes experimentally shown to alter elemental uptake in plants and their orthologs in 10 crop species. When compared to ionomic GWAS data from Arabidopsis, maize, sorghum, soybean, and rice, more than 90% of significant markers are not linked to a KIG gene, indicating the list is far from complete. We expect a large portion of these unknown causal genes to have orthologs in other species, much like our KIG list genes. To find these orthologous genes, we developed a new comparative approach for GWAS. We start with comparable GWAS markers in multiple species, find genes associated with GWAS markers in each species, then compare each species' marker-associated gene list to identify orthologous genes. Preliminary results from testing on ionomic GWAS show this comparative approach finds more trait-linked orthologs in the actual datasets than in 1000 random permutations. Additionally, adding more species to the comparison reduces background noise in the random permutations and retains signal in the actual dataset. Pairing orthologous genes with evidence from analogous GWAS datasets of multiple species produces a prioritized list of conserved candidates in all five species used in this approach- including those with fewer gene annotations. Future research aims to verify ionomic candidates and evaluate candidate prioritizing parameters.

P146

Crop type determines the relation between root system architecture and microbial diversity indices in different phosphate fertilization conditions

(submitted by Sylvia Morais de Sousa Tinoco <sylvia.sousa@embrapa.br>)
Full Author List: Campolino, Mariana L.[1]; Santos, Thiago T.[2]; Lana, Ubiraci G.P.[3]; Gomes, Eliane A.[3]; Guilhen, José H.S.[3]; Pastina, Maria M.[3]; Coelho, Antonio M.[3]; Tinoco, Sylvia M.S.[1 3]
[1] Universidade Federal de São João del-Rei, Praça Frei Orlando, 170, Centro, São João del-Rei – MG, 36307-352, Brazil
[2] Embrapa Agricultura Digital, Avenida André Tosello, 209, Barão Geraldo CP 6041 Campinas – SP, 13083-886, Brazil
[3] Embrapa Maize and Sorghum, Rodovia MG 424 km 45, Zona Rural, Sete Lagoas - MG, 35701-970, Brazil

Synthetic phosphate fertilizers are frequently used in agriculture and their overuse can significantly increase production costs and cause negative environmental impacts. Soil phosphorus (P) availability can be increased by the contribution of the rhizosphere microbiota associated with the plant root system. This work aimed to evaluate the effect of different phosphate fertilization conditions on maize and sorghum genotypes. Four commercial genotypes of maize and four of sorghum were cultivated for two seasons under seven treatments, no addition of P fertilizer, and 50 and 100 kg P_2O_5 ha^{-1} of two rock phosphate (Itafós and OCP) and triple superphosphate. During flowering time, the root system was collected according to the Shovelomics' method and analyzed by a modified version of Digital Imaging of Root Traits (DIRT) system. The modifications made the root system architecture analyses less error-prone and more effective. Moreover, three diversity indices, Shannon-Wiener (H'), Simpson (1-D) and Chao1, were calculated based on the bacteria abundance and richness. The type of crop followed by the genotype and fertilizer were the main factors that affected the root system, grain yield, genetic diversity and abundance of microorganisms. The most productive genotypes had higher root angle and area, increased foraging on the soil surface and P acquisition. Maize presented higher microorganism diversity, root angle and foraging traits while sorghum presented higher abundance of specific taxa, a narrower root angle and smaller foraging. The combined use of less reactive P sources, which could be more soluble over time by the physicochemical processes and soil microbiota activity, together with more efficient genotypes might reduce the amount of soluble phosphate fertilizers applied annually to crops.

Funding acknowledgement: Embrapa (Grant number 12.14.10.003.00.00), CNPq/INCT - 'Plant-Growth Promoting Microorganisms for Agricultural Sustainability and Environmental Responsibility' (Grant number 465133/2014–2, Fundação Araucaria-STI, Capes)

P147

Current applications of neural networks for genomic prediction

(submitted by Karlene Negus <knegus@iastate.edu>)
Full Author List: Negus, Karlene[1,2]; Li, Xianran[3]; Welch, Stephen[4]; Yu, Jianming[1]
[1] Department of Agronomy, Iowa State University, Ames, IA 50011
[2] Interdepartmental Genetics & Genomics, Iowa State University, Ames, IA 50011
[3] USDA-ARS, Wheat Health, Genetics & Quality Research, Pullman, WA 99164
[4] Department of Agronomy, Kansas State University, Manhattan, KS 66506

Genomic prediction is among the ways maize breeding has been able to keep pace with global demand in recent decades. Conventional genomic prediction strategies are regression-based and were designed to make predictions using moderate-to-small sets of genotype data. However, the data landscape has changed since conventional genomic prediction methods were designed and these approaches can be ill-suited for considering and integrating the diverse and evolving data of recent years. For example, characterizing environments (envirotyping) in parallel with genotyping and trait phenotyping for genomic prediction, has gained relevance as the importance of genotype-by-environment interactions is increasingly considered. It remains difficult to integrate genomic, phenomic, and enviromic data via conventional genomic prediction. Additionally, as methods become increasingly high-throughput, alternatives to conventional genomic prediction strategies will be required to better utilize big data. Artificial intelligence-based genomic prediction methods are potential alternatives. Types of artificial intelligence with the capacity to learn, such as neural networks, can better facilitate the translation of large data sets into useful predictions by bypassing the limitations of human expert-driven learning, such as finite time and knowledge. Neural networks are adept at modeling nonlinear processes and handling high-dimensional data. Given these characteristics, neural networks are well suited to the current issues facing genomic prediction. A review of current applications of neural networks for genomic prediction will be described. Topics will include the capability of deep learning to capturing non-additive genetic variation; the use of multi-modal neural networks to integrating various data and model types (i.e., envirotypes, multi-omic data, crop growth models, etc.); potential of neural networks to process genomic prediction relevant data; neuro-symbolic models that combine neural networks with logic-based types of AI to improve the interpretability of neural networks and enhance biological inferences; and the advantages/limitations of these approaches in comparison to conventional strategies.

Funding acknowledgement: NSF Plant Genome Research Program (IOS-2210259), NSF EPSCoR (OIA- 2121410), USDA-NIFA AFRI (2021-67013-33833)

P148

Determining hybrid status using maize leaf reflectance and quantifying spectral plasticity in response to nitrogen treatmentspectral plasticity in response to nitrogen treatment

(submitted by Brooke Bouwens <brookebouwens317@gmail.com>)
Full Author List: Bouwens, Brooke A.[1]; Istipliler, Deniz[2 3 4 5]; Tross, Michael[3 4 5]; Mural, Ravi V.[3 4 5]; Ge, Yufeng[6]; Yang, Jinliang[3]; Schnable, James C.[3 4 5]
[1] Department of Biology-Chemistry, Manchester University, North Manchester, IN, 46962, USA
[2] Department of Field Crops, Ege University, Izmir, Turkiye
[3] Department of Agronomy and Horticulture, University of Nebraska-Lincoln, Lincoln, NE, 68588, USA
[4] Quantitative Life Sciences Initiative, University of Nebraska-Lincoln, Lincoln, NE, 68588, USA
[5] Center for Plant Science Innovation, University of Nebraska-Lincoln, Lincoln, NE, 68588, USA
[6] Department of Biological Systems Engineering, University of Nebraska-Lincoln, Lincoln, USA

Measurements of leaf hyperspectral reflectance have been demonstrated to have the ability to predict numerous crucial plant traits, creating the potential to boost phenotyping accuracy and speed while reducing labor costs. We evaluated the leaf reflectance measurements of 304 maize inbreds from the Backcrossed Germplasm Enhancement of Maize (BGEM) panel and 197 backcrossed hybrids with one BGEM parent grown under two nitrogen treatments at the University of Nebraska-Lincoln in 2022. Measurements spanned the visible, near infrared, and short wave infrared regions. The highest broad-sense heritability values were observed for the wavelengths of 550nm (0.71) and 710nm (0.75) in visible region. The average heritability of individual wavelengths was moderate in the shortwave infrared region (0.40) and low (0.24) in near infrared region. Leaf reflectance could successfully differentiate the hybrids and inbred lines within the dataset (accuracy = 0.84) as well as when testing on external validation datasets (accuracy = 0.75). This ability to classify inbred/hybrid status could prove useful to detect the hybrid rogue plants in maize breeding nurseries which are the products of undesired pollen contamination. Currently, these plants are identified visually by trained maize geneticists, and the availability of trained labor is a significant bottleneck in scaling seed production while ensuring seed purity. The wavelengths 560nm and 710nm are highly responsive to changing nitrogen treatments. Our results suggest that specific regions in leaf reflectance may be significantly correlated with hybrid vigor in maize and also environmental responses of leaf spectrum should be taken into consideration by the researchers in future studies.

Funding acknowledgement: United States Department of Agriculture (USDA)

P149

Drone-based identification of flood-tolerant maize genotypes

(submitted by Madison Mitchell <mnmzbd@umsystem.edu>)
Full Author List: Mitchell, Madison N.[1]; Sidberry, Grace C.[1]; Washburn, Jacob D.[2]
[1] Division of Plant Sciences and Technology, University of Missouri, Columbia, MO 65211
[2] USDA-ARS Plant Genetics Research Unit, USDA-ARS, Columbia, MO 65211

Crop damage from severe weather such as wind, rain, and flooding is projected to cause three billion dollars in crop damage annually by 2030. Accurately assessing these damages and understanding the genetic basis for why some cultivars survive while others do not is challenging, especially for flood damage where the fields may become inaccessible both during and after the flood event. Flooding events are also predicted to increase as climate change brings more extreme weather events. As Unoccupied Aerial Vehicle (UAV or Drone) resources become widespread, phenotyping from the sky has become an option to remove the bottlenecks, challenges, and safety risks of manually measuring flooding while still maintaining or increasing accuracy.
Here we describe the utility of both standard and multi-spectral UAV imaging from before and after an unexpected natural flooding event for characterizing the influence of both environmental (plot location within a field) and genetic factors related to maize flood survival and regrowth. A pipeline for extracting useful phenotypes from pre- and post-flood fields was developed including the creation of orthomosaic images using Pix4D, and the extraction of traits such as Leaf Area Index (LAI), Normalized Difference Vegetation Index (NDVI), and plant height from periods spanning the entire growing season. To assess successful genotypes a comprehensive view of how each variable interacts was visualized and statistically analyzed. This new information will give us a more accurate look into the behavior of maize in flood scenarios and insights into how we might breed for more flood tolerant crops.

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

P150 @NikeeShrestha2

Employing environmental indices to augment cross-environment prediction accuracy in diverse maize populations

(submitted by Nikee Shrestha <nshrestha5@huskers.unl.edu>)
Full Author List: Shrestha, Nikee[1]; Torres-Rodriguez, J. Vladmir[1]; Jin, Hongyu[1]; Newton, Linsey[2]; Thompson, Addie M.[2]; Mural, Ravi V.[1]; Schnable, James C.[1]
[1] Center of Plant Science Innovation & Department of Agronomy and Horticulture, University of Nebraska-Lincoln, Lincoln, NE, USA
[2] Plant Resilience Institute & Department of Plant Soil and Microbial Sciences, Michigan State University, East Lansing, MI, USA

Interactions between genetic and environmental factors produce a diversity of phenotypic outcomes across different environments. Many approaches to studying genotype x environment interaction depend on trait data collected from the target environment. While this approach can be powerful for identifying genes controlling phenotypic plasticity, in many contexts it is important to be able to predict how plants will respond to previously unobserved environments, particularly environments in future years. Here we evaluated a recently developed approach Critical Environmental Regressor through Informed Search (CERIS) – Joint Genomic Regression Analysis (JGRA) algorithm for identifying an environmental index (i.e., a combination of an environmental factor and growth period) which can be used to predict plant phenotypes in previously observed as well as unobserved environments. We employed this approach on flowering time for 670 genotypes of the expanded Wisconsin Diversity Panel grown across four different environments (Michigan and Nebraska for two consecutive years; 2020 and 2021 respectively). We identified an environmental index; growing degree days from 43 to 50 days after planting that explains 99.2% variation in the average flowering time across four environments. Environmental index was then used to estimate the environmental mean in unobserved environments. The prediction accuracy for flowering time of tested genotypes in an unobserved environment was 0.93. The prediction accuracy for flowering time of untested genotypes in an observed environment was 0.84. The prediction accuracy for flowering time of untested genotype in an unobserved environment was 0.83. The findings from this evaluation of the CERIS-JGRA in a large maize population validates the generalizability of this approach. Cross validation under multiple scenarios which exceed the prediction accuracy obtained when using solely genomic information suggest that approaches like CERIS-JGRA can facilitate accurate prediction of other yield determining traits and potentially yield itself, across multiple and future environments.

Funding acknowledgement: National Science Foundation (NSF)

P151

Evaluation of hybrid maize expressing flavonoids for fall armyworm control

(submitted by Bryan Panek <bpp122@psu.edu>)
Full Author List: Panek, Bryan[1]; Lesko, Tyler[1]; Beuzelin, Julien[3]; Peiffer, Michelle[2]; Felton, Gary[2]; Chopra, Surinder[1]
[1] Department of Plant Science, The Pennsylvania State University, University Park, PA 16802, USA
[2] Department of Entomology, The Pennsylvania State University, University Park, PA 16802, USA
[3] Everglades Research & Education Center, University of Florida Institute of Food and Agricultural Sciences, Belle Glade, FL 33430, USA

Fall armyworm (FAW, Spodoptera frugiperda (J.E. Smith)) is an invasive pest that has spread around the world since it was first detected in West Africa in 2016. A polyphagous feeder, FAW consumes over 350 plant hosts but exhibits preference for grasses, including crops such as rice, sorghum, and maize. FAW has been controlled predominantly with the application of insecticides and adoption of transgenic Bt (Bacillus thuringiensis) hybrid maize. However, FAW is in the top 15 insect species that has developed field evolved resistance to many insecticides. A sustainable alternative to traditional and transgenic control methods can be breeding maize hybrids producing compounds conferring resistance to FAW larvae. 3-Deoxyanthocyanidins (3-DAs) are flavonoids produced by maize and sorghum that have shown inhibition of FAW larval growth and increased mortality when compared to controls. Attributed to the phenylpropanoid pathway, biosynthesis of 3-DAs is completed by using naringenin as a deciding point before forking off to synthesize many other flavonoids. Naringenin also gives rise to multiple flavonoids with tissue specific pigmentation patterns in maize and those compounds have been used as phenotypic markers in genetic studies. We have developed maize lines and hybrids that endogenously produce multiple flavonoid pigments in leaves and have the potential to reduce the devastating impact of FAW feeding. In this project, I used different maize near isogenic lines (NILs), flavonoid mutants, and hybrids to evaluate FAW damage, impact on grain yield, and efficacy in increasing mortality and inhibiting larval growth.

Funding acknowledgement: United States Department of Agriculture (USDA)

P152

Exploring the genetic determinants of phenotypic means and plasticity for flowering time in a Maize Association Panel

(submitted by Ravi Mural <rmural2@unl.edu>)
Full Author List: Mural, Ravi V.[1]; Grzybowski, Marcin[1]; Sun, Guangchao[1]; Zhongjie, Ji[2]; Thompson, Addie M.[2]; Schnable, James C.[1]
[1] Center for Plant Science Innovation & Department of Agronomy and Horticulture, University of Nebraska-Lincoln, Lincoln, NE, USA
[2] Plant Resilience Institute & Department of Plant Soil and Microbial Sciences, Michigan State University, East Lansing, MI, USA

The timing of flowering in maize is subject to complex regulation that depends on genotypic factors, environmental factors, and the interaction between the two. A better understanding of the genes controlling the plasticity of specific phenotypes in response to environmental changes would aid in predicting how crop plants will perform in previously untested environments, including future environments. In this study, we sought to identify specific loci controlling mean flowering time as well as linear and non-linear plasticity across three flowering time related traits measured for an approximately 750 entry maize population tested in four environments. Trait means, linear plasticity and non-linear plasticity were quantified using Bayesian Finlay-Wilkinson regression analysis. A set of 136 unique peaks, capturing 273 marker trait associations using a suggestive cutoff were identified using ~26M markers in a resampling based GWAS. Of these, a subset of 47 peaks capturing 95 marker trait associations were retained at a more stringent cutoff. Among confident peaks, 22 were associated solely with main effects, one peak was associated with both main effects and linear plasticity, while 24 peaks were specific for only linear plasticity. A set of previously published flowering time traits were used to evaluate the relationship between mean/plasticity GWAS signals and those identified by conventional analyses. Often the conventional hits overlapped with the main effects and rarely overlapped with the hits associated with linear plasticity. A set of 33 known flowering time genes exhibited substantial overlap with both main effect peaks from Bayesian Finlay-Wilkinson regression and peaks from single environment GWAS. Linear plasticity peaks were not significantly associated with previously published genes linked to flowering time in maize. High confidence and large effect peaks associated with linear plasticity suggest that currently, uncharacterized genes play key roles in determining the response of maize to new environments.

Funding acknowledgement: National Institutes of Health (NIH), United States Department of Agriculture (USDA), Department of Energy (DOE), Advanced Research Projects Agency–Energy

P153

Fine-mapping efforts reduced chromosomal region responsible for qshgd1 in maize

(submitted by Tyler Foster <tfoster@iastate.edu>)
Full Author List: Foster, Tyler L[1]; Kloiber-Maitz, Monika[2]; Gilles, Laurine[3]; Frei, Ursula K[1]; Lubberstedt, Thomas[1]
[1] Department of Agronomy, Iowa State University, Ames, IA, 50011, USA
[2] KWS SAAT SE & Co. KGaA, Grimsehlstr. 31, Einbeck, 37574, Germany
[3] Limagrain Europe SAS, Research Centre, 63720, Chappes, France

Inbred lines A427 and Wf9, the former displaying high spontaneous haploid genome doubling (SHGD) and the latter displaying low SHGD, were selected to develop a mapping population to investigate a large-effect QTL - qshgd1. The limiting factor for SHGD, haploid male fertility (HMF), was the primary trait targeted in this study. The need for this study was highlighted by research findings that suggest a novel mapping population could help in narrowing the chromosome region in which this important QTL is located. Additionally, utilization of SHGD has the potential to increase the rate of genome duplication beyond the scope of traditional methods that utilize chemical duplication agents. A total of 30 novel recombinant groups were derived utilizing 17 markers that spanned a 45 Mb region, that includes an inversion and centromeric region, on chromosome 5. Recombinant groups were grown in three unique environments to study HMF, where correlations for HMF scores across environments were highly significant. In addition, the haploid recombinant groups showed differing levels of restored male fertility, with a few recombinant groups exhibiting high HMF. However, a large “GAP” region with suppressed recombination demonstrated it is more challenging to locate the exact location of this QTL than initially anticipated. Overall, qshgd1 must be examined further, possibly with methods outside traditional mapping techniques, and this study shows data trends that support further research is still needed to develop a better understanding of the genetic basis of SHGD.

Funding acknowledgement: National Science Foundation (NSF), United States Department of Agriculture (USDA), Iowa State University Plant Sciences Institute, Iowa State University Raymond F. Baker Center for Plant Breeding, Limagrain, KWS

P154 @freeaSarahgene

Free asparagine in staple crops and its affinity to form acrylamide

(submitted by Sarah Oliver <sloxmd@umsystem.edu>)
Full Author List: Oliver, Sarah L.[1]; Shrestha, Vivek[2]; Jenkins, Lauren M.[1]; Angelovici, Ruthie[1]; Flint-Garcia, Sherry A.[1 3]
[1] Division of Biological Sciences, University of Missouri, Columbia, Missouri, United States 65201
[2] Bayer Crop Sciences, St. Louis, Missouri, United States 63167
[3] Plant Genetics Research Unit, USDA-ARS, Columbia, Missouri 65201

Asparagine, in its free form, is directly correlated with the formation of the carcinogen acrylamide in many staple crops. Food products high in free asparagine and starch, cooked under hot and dry conditions such as baking or frying, are primed for acrylamide accumulation. Due to variation in diets across cultures, socio-economic status, and life stage, the level of threat that acrylamide poses on the average human is unknown. However, recent data is suggesting that dietary acrylamide exposure is playing a much larger role in the incidence of cancer than previously thought. While reducing free asparagine could be a logical step towards finding a solution to this problem, it is important to note that free asparagine is critical in germination, stress response, and other processes in a variety of plants. Metabolism of this amino acid is largely dependent on environmental conditions and the genetic controls and is highly variable between species. Despite this, incredible advances have been made in reducing acrylamide formation potential in a few staple crops. Unfortunately, there is little to no data to help work towards a solution in many of the highest-potential crops including maize. In this review, we delve into the influences on free asparagine metabolism throughout plant growth and development, summarize the work done to minimize acrylamide formation potential in several staple crops, and discuss next steps in maize.

Funding acknowledgement: United States Department of Agriculture (USDA)

P155

Genetic characterization of a potentially new fertilization barrier system with unlinked male and female controls

(submitted by Destiny Tyson <dntyson@ncsu.edu>)
Full Author List: Tyson, Destiny[1]; Kermicle, Jerry[2]; Chen, Qiuyue[3]; Rellan-Alvarez, Ruben[4]; Holland, James[3 5]
[1] Department of Biological Sciences, North Carolina State University, Raleigh, NC, USA
[2] Department of Genetics, University of Wisconsin, Madison, WI, USA
[3] Department of Crop and Soil Sciences, North Carolina State University, Raleigh, NC, USA
[4] Department of Molecular and Structural Biochemistry, North Carolina State University, Raleigh, NC
[5] USDA-ARS, Raleigh, NC, USA

Many plant species have genetically controlled self-incompatibility systems that prevent self-fertilization by distinguishing between self vs. non-self pollen. These systems promote outcrossing and limit inbreeding. Cross-incompatibility systems have been described in maize and may have evolved as selfish genes or to prevent the production of unfit inter-population hybrids between maize and its wild relatives, teosintes. Cross-incompatibility also has practical uses in modern maize by protecting organic and specialty corn, such as popcorn and some sweet corn, from being contaminated by undesirable pollen. Pollen-pistil compatibility governs both self and cross-hybridization. To date, three cross incompatibility systems have been identified in maize and teosinte, namely *teosinte crossing barrier 1* (*Tcb1*), *gametophyte factor 1* (*Ga1*), and *gametophyte factor 2* (*Ga2*). We recently identified a teosinte-derived line of maize inbred W22 that sets seed poorly following self-pollination. Relative to the standard inbred, poor seed set is inherited as a major, recessive gene. Poor set also occurs when this line was pollinated with most other maize lines, whereas good set followed pollination with the majority of the few teosinte accessions tested. Restoration of compatibility by teosinte appears to be simply inherited and to assort independently of incompatibility as though separate female and male loci determine pollination success. For present use, these factors are designated as "*unlinked pollination barrier, female"* and *"unlinked pollination barrier, male*". We are analyzing seed set phenotype and genotyping an F2 population that segregates for the female factor and a backcross-F1 population assorting for the male factor to map the genes involved. It is not known whether genes controlling this fertilization barrier are allelic to previously described cross-incompatibility genes and whether, within teosinte, this system serves mainly to govern cross or self-incompatibility.

P156

Genetic dissection using nested-association mapping (NAM) reveals quantitative trait loci (QTL) conferring resistance to Tar Spot in maize.

(submitted by Raksha Singh <raksha.singh@usda.gov>)
Full Author List: Singh, Raksha[1]; Crane, Charles[1]; Shim, Sujoung[2]; Telenko, Darcy[2]; Goodwin, Stephen[1]
[1] Crop Production and Pest Control Research Unit, U.S. Department of Agriculture-Agricultural Research Service (USDA-ARS), 915 W State St, West Lafayette, IN 47907
[2] Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN 47907

Tar spot, caused by the obligate fungal pathogen *Phyllachora maydis*, constitutes a major threat to corn production in North America, the Caribbean, Central and South America. Apart from a significant quantitative trait locus (QTL) on chromosome 8 that was mapped in tropical maize line populations, little is known regarding the genetics of maize resistance to *P. maydis*. Therefore, more research is required to identify additional sources of resistance against *P. maydis* populations in North America. Previously, we evaluated 26 parental inbred lines from the Nested Associated Mapping (NAM) population for tar spot resistance in Indiana in replicated field trials under natural infection. Our findings showed that inbred B73, the common parent of the NAM populations, was moderately susceptible. However, resistant inbred lines NC358, CML103, CML52, CML322, M162W, CML228, CML333, TZI8 and KI11, provided a potential germplasm source for genetic mapping of *P. maydis* resistance in maize. Next, we employed the B73 × CML52 recombinant-inbred line (RIL) population to identify major quantitative trait loci for *P. maydis* resistance. A panel of 197 RIL lines derived from a cross of B73 × CML52 was used to score tar spot disease phenotypes. Association mapping resulted in the detection of seven QTL on chromosomes 1, 2, 4, 5, 7, 8, and 9, including a major QTL on chromosome 9 and another moderately strong QTL on chromosome 2. Collectively, our findings revealed a novel QTL on chromosome 9 (qTL9) associated with *P. maydis* resistance. Future investigation involves the validation of qTL9 by simple-sequence repeat (SSR) marker analysis and the identification of candidate genes in the qTL9 region by differential gene expression analysis, which will provide insightful information to improve *P. maydis* resistance in maize.

Funding acknowledgement: United States Department of Agriculture (USDA)

P157

Genetics of leaf angle across canopy levels in maize

(submitted by Boris M. E. Alladassi <aboris@iastate.edu>)
Full Author List: Alladassi, Boris M. E.[1]; Schoenbaum, Gregory R.[1]; Li, Xianran[2]; Yu, Jianming[1]
[1] Department of Agronomy, Iowa State University, Ames, IA 50011
[2] USDA-ARS, Wheat Health, Genetics, and Quality Research, Pullman, WA 99164

Leaf angle (LA) is an important canopy architecture trait for high planting densities. Accessions of maize exhibit different LA architectures that affect their light interception efficiency and photosynthetic activity. Here, we mapped the genomic regions controlling LA variation at four canopy levels (lower, middle, mid-upper, and upper) in a set of 309 maize doubled haploid (DH) lines evaluated across three growing seasons. The DH lines were developed by crossing B73 and Mo17 with PHW30, three inbred lines that represent the major maize heterotic groups in the US and have distinct LA architectures. Hierarchical clustering of LA measurement across the four canopy levels classified the DH lines into six clusters. Three clusters had dynamic LA across canopy levels while the other three had low, medium, and high LA throughout the canopy. There was a moderate to strong correlation (r = 0.31-0.75) among the four canopy levels, and 80-92% of the phenotypic variance observed was due to genetic effects. Composite interval mapping of LA at individual canopy levels detected five QTLs on chromosomes 1, 3, 4, and 5 controlling LA variation consistently across two to three canopy levels and one QTL on chromosome 7 specific to the upper canopy level. Next, we used a multivariate QTL mapping approach to increase the statistical power and refine the QTLs' locations. The multivariate analysis detected a new QTL on chromosome 1 and confirmed all previous six QTLs. A QTL on chromosome 3 had the strongest (LOD score > 17) peak and co-localizes with the maize gene *liguleless2* known to control LA variation. These results have improved our understanding of consistent and canopy-level-specific loci controlling LA in maize. Gene expression analysis is underway on a representative sample of 10 DH lines to dissect further the genetic control of dynamic LA observed across canopy levels in maize.

Funding acknowledgement: National Science Foundation (NSF), Iowa State University Plant Science Institute, Raymond F. Baker Center for Plant Breeding, Iowa Crop Improvement Association

P158

Genome-wide association studies to establish the genetic basis of agro-mophological and climatic traits in wild maize relatives

(submitted by Joseph Atemia <j.atemia@fz-juelich.de>)

Full Author List: Atemia, Joseph[1]; Piñero, Daniel[2]; Sánchez, José de Jesús[3 4]; Rodríguez, Diana[5 6]; Larios, Lino de la Cruz[3]; Acevedo, Francisca[7]; Wegier, Ana[2 8]; Galvez, Nancy[2]; Usadel, Björn[1]; Mastretta-Yanes, Alicia[7]; Hallab, Asis[1 9]

[1] Institute of Bio- and Geosciences - Bioinformatics (IBG-4) - Forschungszentrum Jülich, Germany
[2] Instituto de Ecologia, UNAM, Mexico
[3] Centro Universitario de Ciencias Biológicas y Agropecuarias, Guadalajara, Mexico
[4] Universidad de Guadalajara, Guadalajara, Mexico
[5] Instituto Tecnológico de Tlajomulco, Jalisco, Mexico
[6] Tecnológico Nacional de México, Jalisco, Mexico
[7] Comision Nacional para el Conocimientoy Uso de la Biodiversidad- Consejo Nacional de Ciencia y Tecnología, Mexico
[8] Jardín Botánico, Mexico
[9] Technische Hochschule Bingen, Germany

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, the Mexican partners performed an extensive teosinte sampling, including ca. 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 yielding ca. 30,000 SNPs. Ecogeographical analyses were also performed. 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). We report candidate genes contained or in close proximity to SNPs associated with these traits. In-depth results as well as an outlook on future genome and transcriptome sequencing and sequencing analysis directed at the identification of loci linked with adaptations to extreme growing conditions will be presented in this talk.

Funding acknowledgement: Bundesministerium für Bildung und Forschung

P159

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

(submitted by Jacob Hinrichsen <jhinrich@iastate.edu>)
Full Author List: Yu, Jianming[1]; Tang, Lie[2]; Negus, Karlene[1]; Alladassi, Boris M.E.[1]; Li, Dongdong[1]; Schoenbaum, Gregory R[1]; Hinrichsen, Jacob T[1]; Raj, Aditya[2]; Liu, Xuan[2]; Butler, Nathan[2]; Li, Xianran[3]; Bang-Jensen, Valerie[4]; Lubkowitz, Mark[4]; Yang, Bing[5]; Scanlon, Michael J[6]
[1] Department of Agronomy, Iowa State University, Ames, IA, USA
[2] Department of Agricultural and Biosystems Engineering, Ames, IA, USA
[3] USDA-ARS, Wheat Health, Genetics, and Quality Research Unit, Pullman, WA, USA
[4] Saint Michael's College, Colchester, VT, USA
[5] Division of Plant Sciences, University of Missouri, Columbia, MO, USA
[6] Plant Biology Section, School of Integrative Plant Science, Cornell University, Ithaca, NY, USA

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 preliminary 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 have 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: Laser-Microdissection RNA sequencing analyses of genes underlying leaf angle variation across the canopy. Aim 3: Functional analyses of genes underlying leaf angle variation through CRISPR/Cas9-based gene editing in two maize inbreds. Aim 4: Develop educational materials for K-12 teachers to create teaching gardens that help incorporate plant biology content into their curriculum. In addition to cross-training of postdocs and students, we will specifically develop and disseminate grade-level text sets and accompanying seeds for the creation of teaching gardens on school grounds.

Funding acknowledgement: National Science Foundation (NSF)

P160

Genomic prediction across two breeding cycles in maize silage using a factorial approach

(submitted by Alizarine Lorenzi <alizarine.lorenzi@inrae.fr>)
Full Author List: Lorenzi, Alizarine[1]; Bauland, Cyril[1]; Mary-Huard, Tristan[1 2]; Pin, Sophie[1]; Palaffre, Carine[3]; Guillaume, Colin[4]; Lehermeier, Christina[5]; Charcosset, Alain[1]; Moreau, Laurence[1]
[1] Université Paris-Saclay, INRAE, CNRS, AgroParisTech, Génétique Quantitative et Evolution - Le Moulon, 91190 Gif-sur-Yvette, France
[2] MIA, INRAE, AgroParisTech, Université Paris-Saclay, 75005, Paris, France,
[3] UE 0394 SMH, INRAE, 2297 Route de l'INRA, 40390, Saint-Martin-de-Hinx, France
[4] Maïsadour Semences SA, F-40001 Mont de Marsan Cedex, France
[5] RAGT2n, Genetics and Analytics Unit, 12510 Druelle, France

Genomic selection enables the prediction of all possible single-crosses between candidate lines by using a training set composed of genotyped and phenotyped individuals to calibrate an equation of prediction. The design used as training set, its size, composition and relationship with the set of validation can affect prediction accuracies. Previous simulation and experimental studies have shown the potential of using a sparse factorial design instead of tester designs as training set. This work aimed at: (i) evaluating the efficiency of a factorial training design in the context of prediction across breeding cycles and (ii) investigating optimization strategies to construct the training set. The study relies on two breeding cycles issued from a multiparental connected reciprocal population generated from the flint and dent complementary groups. In each group, about 800 inbred lines were generated and evaluated for their hybrid value in different factorial designs for their silage performance. The best 30 lines from each group were selected based on genomic predictions and intercrossed to produce the next generation evaluated in a factorial design. We showed that using the first cycle to predict the next one gave good predictive abilities, reaching 0.6 for dry matter yield. By adding hybrids from the new cycle to the training set, we increased predictive ability by 0.1 for dry matter yield. This indicates the benefit of recalibrating across breeding cycles. To optimize the training set and determine the interest of recalibrating model by phenotyping part of the G1 hybrids, a criterion maximizing the mean of the expected reliabilities (CDmean) was used. Samples chosen based on CDmean gave higher predictive abilities than random samples for various calibration set sizes. Our results confirm the potential of sparse factorial designs for revisiting genomic hybrid breeding schemes.

Funding acknowledgement: Lidea, Limagrain Europe, Maïsadour Semences,Corteva, RAGT2n,Syngenta seeds, KWS,ANRT

P161

Genomic selection models for untested maize hybrids performance prediction in non-evaluated environments

(submitted by Jenifer Camila Godoy dos Santos <godoycamilajds@usp.br>)
Full Author List: Godoy dos Santos, Jenifer Camila[1 2]; De Leon Gatti, Natalia[1]; F. Garcia, A. Augusto[2]
[1] University of Wisconsin; Madison; Wisconsin; United of States of America
[2] University of Sao Paulo; Piracicaba, Sao Paulo; Brazil

Genomic Selection (GS) is a promising approach to simultaneously predict individual breeding values using thousands of genome markers distributed throughout the genome. Suitable GS models provide accurate predictions even for untested genotypes. For this reason, this tool has great potential to reduce phenotyping costs, reduce the breeding cycle, and increase the genetic gain per unit of time. The breeding values are generated by considering allelic substitution effects, including the markers' additive and dominant effects. Both effects can contribute substantially to the variation of complex traits in plant breeding programs. However, most GS models have primarily focused on estimating the additive effects. Genotype-by-Environment Interaction (GEI) is another essential factor in GS studies since many studies have shown that GS models incorporating GEI have higher predictive ability. In this context, the primary purpose of our research is to develop GS models that simultaneously incorporate additive, dominance, and GEI effects for predicting the performance of maize hybrids for grain yield in non-evaluated environments. For that, we are using grain yield data from 156 maize hybrids evaluated in two different crop seasons, at nine locations and under two management conditions in Brazil. Of the 156 hybrids, 149 are single-crosses, represented by three heterotic groups: dent, flint, and the so-called group C. The other hybrids include four common checks (commercial maize cultivars), one double-cross, and two three-way crosses. DNA from the 144 maize inbred lines, used as progenitors of the single-cross hybrids, was extracted from young leaves and genotyped using the standard protocol of Genotyping-by-Sequencing (GBS). We inferred the hybrids' genotypes based on their parents' genotypes using single-nucleotide polymorphism markers obtained via GBS. We will use four cross-validation schemes: CV1, CV2, CV0, and CV00 to assess the predictive capacity of the proposed models.

Funding acknowledgement: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES); Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq); Empresa Brasileira de Pesquisa Agropecuária (Embrapa); University of Wisconsin (UW-Madison)

P162 @spklein2

Genotype-specific differences in the transcriptional landscape underlie varying root phenotypes under nitrogen stress

(submitted by Stephanie Klein <spklein@iastate.edu>)
Full Author List: Klein, Stephanie P.[1]; Anderson, Sarah N.[1]
[1] Department of Genetics, Developmental & Cell Biology; Iowa State University; Ames, IA, 50011

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 SNPs as a means 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. RNA-seq enables us to evaluate differential gene expression along with genomic sequences as a direct readout of novel transcriptional responses to stress. Using the maize NAM founders, we sought to identify structural variants associated with root growth angle, a root architectural trait that has been directly linked to improved nitrogen (N) acquisition. We planted the NAM founders in the field under high and low N and observed substantial variation in root system architecture and plant performance measures under N-stress. We identified two genotypes that warranted further investigation because of their contrasting responses to N-stress: B73, which was steep-angled and non-responsive to stress, and Oh7B, whose root growth angle became significantly steeper under N-stress. Using RNA-seq, we evaluated transcriptional changes to N-stress in these two genotypes across three nodal root tissues in the greenhouse. Preliminary analyses revealed that the zone nearest the root tip of Oh7B contained nearly twice as many differentially expressed genes as that of B73. We plan to evaluate these differences at the network scale to identify genes contributing to this differential N-responsiveness and find regulatory regions, particularly those in transposable elements, potentially regulating gene activity. Ultimately, we will integrate these efforts to associate genomic structural variants with root growth angle phenotypes, thereby enabling us to bridge the gap between genome and phenome.

Funding acknowledgement: United States Department of Agriculture (USDA)

P163

Grain yield genetic gain for Bayer Crop Science maize hybrids

(submitted by Kyle King <kylek@iastate.edu>)
Full Author List: King, Kyle[1]; Trifunovic, Slobodan[2]; Eudy, Doug[2]; Lamkey, Kendall[1]; Topp, Chris[3]; Vyn, Tony[4]; Archontoulis, Sotirios[1]
[1] Iowa State University, Department of Agronomy, Ames, IA, US
[2] Bayer Crop Sciences, Chesterfield, MO, US
[3] Donald Danforth Plant Science Center, Olivette, MO, US
[4] Purdue University, Department of Agronomy, West Lafayette, IN, US

We performed experiments across multiple environments to determine Bayer's Crops Science grain yield genetic gain for maize hybrids released from 1980 to 2020. We studied 40 maize hybrids of 103-day relative maturity (n=16 site-years) and 40 maize hybrids of 111-day relative maturity (n=18 site-years) in the US Corn Belt. Our results revealed that the genetic gain for grain yield has increased at a faster rate for 111-day hybrids (1.84 bu/ac/yr) compared to 103-day hybrids (1.58 bu/ac/yr). To understand why the newer hybrids yield better than the order ones and also the genetic gain differences among maturity groups, we examined grain yield components, namely kernels per unit area (established around mid-growing season, silking) and the individual kernel weight (established during the grain filling period). We found that the yield of the newer hybrids is increasing because both of the increase in kernel number per unit area and also because of the increase in kernel weight. In the 111-day hybrids, the kernel number contributed 43% of the yield while the kernel weight 57% of this increase. In the 103-day hybrids, the kernel number contributed 70% of the yield increase while the kernel weight 30% of this increase. We concluded that the increase in kernel number is the main reason for the grain yield increase in 103-day hybrids and the increase in kernel weight is the main reason for the grain yield increase in 111-day hybrids.

Funding acknowledgement: FFAR, Bayer Crop Science

P164

Harvest index has increased over the last 50 years of maize breeding

(submitted by Alejo Ruiz <aruiz@iastate.edu>)
Full Author List: Ruiz, Alejo[1]; Trifunovic, Slobodan[2]; Eudy, Douglas M.[2]; Sciarresi, Cintia S.[1]; Baum, Mitchell[1]; Danalatos, Gerasimos J.N.[1]; Elli, Elvis F.[1]; Kalogeropoulos, Georgios[1]; King, Kyle[1]; dos Santos, Caio[1]; Thies, August[3]; Olmedo Pico, Lia[4]; Castellano, Michael J.[1]; Schnable, Patrick S.[1]; Topp, Christopher[3]; Graham, Michael[2]; Lamkey, Kendall R.[1]; Vyn, Tony J.[4]; Archontoulis, Sotirios V.[1]
[1] Iowa State University, Department of Agronomy, Ames, IA, US
[2] Bayer Crop Sciences, Chesterfield, MO, US
[3] Donald Danforth Plant Science Center, Olivette, MO, USA
[4] Purdue University, Department of Agronomy, West Lafayette, IN, US

Historically, the harvest index (HI, the fraction of above-ground biomass allocated to grain yield) has been assumed to be constant for maize over the years. This study tests the hypothesis that HI has increased over a century of maize breeding by reporting new data from a comprehensive experiment in the US Corn Belt and synthesizing the new findings with literature data. We studied 54 commercial hybrids (103-day and 111-day relative maturities) released from 1983 to 2020 across 13 environments, including plant density and N-fertilizer treatments. Results showed that HI has increased over the years from 0.516 to 0.571 in 103-day hybrids and from 0.537 to 0.584 in 111-day hybrids. The genetic gains were similar across environments and management treatments, indicating that this increase is attributed to maize breeding. Our results, combined with 16 literature datasets, revealed a 0.26% $year^{-1}$ relative increase in HI since 1964. We estimated that the increase in HI accounts for ca. 15% of the historical maize yield increase in the US Corn Belt over the past 50 years. Our findings enhance our knowledge of maize HI, will support robust estimations of carbon inputs in sustainability studies, and inform crop models to better capture historical yield increases.

Funding acknowledgement: FFAR, Bayer Crop Science

P165 @melissa_draves

Heirloom maize of the United States and Canada

(submitted by Melissa Draves <madcfr@umsystem.edu>)
Full Author List: Draves, Melissa A.[1]; Hall, Garret[1]; Washburn, Jacob D.[2]; Thompson, Addie[3]; Gage, Joseph[4]; Holland, James[5]; Flint-Garcia, Sherry[2]
[1] University of Missouri, Division of Plant Science and Technology, Columbia MO 65211
[2] USDA-ARS Plant Genetics Research Unit, Columbia MO 65211
[3] Michigan State University, Plant, Soil, and Microbial Sciences Department, East Lansing MI 48824
[4] North Carolina State University, Department of Crop and Soil Sciences, Raleigh NC 27695
[5] USDA-ARS Plant Science Research Unit, Raleigh NC 27695

Hybrid maize has been a long standing crop in the agricultural world, making it a necessity for livestock feed and fuel production. However, little focus is given to landrace/heirloom maize that was primarily utilized for food and production needs before the advent of modern hybrids. Heirloom maize has enormous levels of genetic diversity, making it an interesting foundation for future breeding programs. Due to its lack of systematic improvement by humans, heirloom maize is of particular interest to organic and smallholder farms, restaurants, and chefs, creating a niche market willing to utilize these products. While heirloom maize of Europe, South America, and Mexico has been extensively studied, the heirlooms of North America and Canada have yet to be fully described. This project will focus on 1000 heirlooms collected from the United States and Canada, with the goal of creating an expansive phenotypic and genotypic data set that can be utilized for culinary and breeding practices. Plants will be phenotyped using manual and automated technologies with the goal of collecting in-field plant traits, ear traits, and kernel traits. Kernels will be analyzed using NIR to estimate seed composition traits. Genotyping of a core set of lines will be accomplished using Pool-seq genotyping that will also help to elucidate population structure and distinguish varieties with similar names and/or morphological traits. GWAS using phenotypic, genotypic, and historical weather data will be conducted to obtain key adaptation and morphological genes. In the future, a subset of populations will be chosen for improvement in order to investigate if varieties are able to withstand selection and be released to market. Overall, this project aims to create a comprehensive data set of US and Canadian heirlooms that can be utilized for line improvement, identification of adaptation and morphological genes, and potential applications beyond conventional breeding for yield.

Funding acknowledgement: United States Department of Agriculture (USDA), Life Sciences Fellowship

P166

High-throughput mutant screening using unoccupied aerial systems

(submitted by Piyush Pandey <piyush.pandey@usda.gov>)
Full Author List: Pandey, Piyush[1]; Best, Norman B.[1]; Washburn, Jacob D.[1]
[1] United States Department of Agriculture, Agricultural Research Service, Columbia, MO, 65211, USA

Maize mutant populations have long served as a valuable forward genetics resource. Many important discoveries related to the genetic control of agriculturally important traits have been made through forward genetic screens and their follow up studies. The creation and screening of new mutagenized populations is sure to yield more useful genetic variation and provide the fodder for further research and discovery. The larger the number of mutants that can be screened the more likely the possibility of finding useful mutations. However, the manual screening of mutant populations is a labor intensive bottle neck that limits screening numbers.
In this project we are developing tools, methods, and pipelines for using Unoccupied Aerial Systems (UASs, UAVs, or Drones) to enable the semi-automated screening of large mutant populations in a field setting. Current UAS methods are mainly designed for analysis at the field or plot level and tools for single plant analysis (as is often desirable with mutation screens) are lacking. We use object detection models based on deep Convolutional Neural Networks (CNNs) to detect individual maize plants in UAS images followed by the extraction of individual plant phenotypes. Depending on the UAS sensor systems used, traits including color-based indices, canopy coverage, canopy temperature, plant height, and others can be derived.

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

P167

High-throughput phenotyping of phyllotaxy via 3D reconstruction enables quantitative genetic analysis of canopy architecture

(submitted by Jensina Davis <jdavis132@huskers.unl.edu>)
Full Author List: Davis, Jensina M.[1 2]; Tross, Michael C.[1 2]; Grove, Ryleigh J.[1]; Gaillard, Mathieu[3]; Benes, Bedrich[3]; Schnable, James C.[1 2 4]
[1] Center for Plant Science Innovation, University of Nebraska-Lincoln, Lincoln, NE 68504, USA
[2] Complex Biosystems Graduate Program, University of Nebraska-Lincoln, Lincoln, NE 68504, USA
[3] Department of Computer Science, Purdue University, West Lafayette, IN 47907, USA
[4] Department of Agronomy and Horticulture, University of Nebraska-Lincoln, Lincoln, NE 68504, USA

Canopy architecture traits influence the light and water use efficiencies of multiple grain crop species. The role of leaf angle in determining canopy architecture has been comparatively well studied, and an increase in leaf angle (e.g. more erect leaves) is associated with the increased tolerance of modern maize hybrids to high planting density. However, additional traits also contribute to leaf canopy architecture including leaf number, leaf length/width, and phyllotaxy (leaf arrangement around the stem). Of these traits, phyllotaxy is perhaps the most challenging to phenotype in a quantitative fashion across large numbers of plants. In computational models and digital twins of maize and related species, phyllotaxy has generally been assumed to be stable and distichous, with an angle of 180 degrees between sequential leaves. Here we use a voxel-carving based approach to generate 3D reconstructions of plants from multiple 2D images and demonstrate heritable (e.g. explained by differences between genotypes) variation in this quantitative trait across an association mapping population in sorghum, a close relative of maize. Phyllotaxy is a model for the important traits which may previously have been under studied as a result of the difficulty and time-consuming nature of manual measurements but which are becoming practical to score across large populations via 3D reconstruction and imaging. The method we present here has the potential to enable quantitative genetics studies of genes controlling phyllotaxy in maize and sorghum as well as enabling breeding to redesign and further optimize canopy architecture.

Funding acknowledgement: United States Department of Agriculture (USDA), Department of Energy (DOE), Foundation for Food and Agriculture Research

P168

Identification and characterization of transcription factors regulating aquaporin gene expression

(submitted by Maxime Laurent <maxime.laurent@uclouvain.be>)
Full Author List: Laurent, Maxime J.[1]; Maistriaux, Laurie[1]; Duborjal, Hervé[2]; Rincent, Renaud[3]; Nicolas, Stéphane[3]; Chaumont, François[1]
[1] Louvain Institute of Biomolecular Science and Technology, UCLouvain, Louvain-la-Neuve, Belgium
[2] Limagrain Europe, Centre de recherche de Chappes, Chappes, France
[3] Université Paris-Saclay, INRAE, CNRS, AgroParisTech, GQE-Le Moulon, Gif-sur-Yvette, France

Aquaporins (AQP) are channels that modulate the permeability of cell membranes to water and other small solutes. In plants, they are involved in numerous physiological and cellular processes, including hydraulic conductivity of cells and tissues, cell expansion and homeostasis. Given their involvement in such essential roles, they are tightly regulated at multiple levels according to developmental stages, organs, daytime, and in response to environmental stimuli. We are mainly interested in their transcriptional regulation, for which expression profiles under various conditions have been reported, but little data is available on the specific molecular components involved in their transcription.Our goal is to uncover mechanisms that regulate the transcription of *AQP* genes in maize leaves in well-watered (WW) and water deficit (WD) conditions. Based on 3' RNA-seq data from mature zone (MZ) samples from 254 maize lines (DROPs panel) growing under WW and WD conditions, we identified the *AQP* genes that are expressed in the MZ and how their expression varies depending on soil water availability. In addition, we performed a genome-wide association study (GWAS) and mapped expression quantitative trait loci (eQTL) for the *AQP* genes expressed in MZ under both hydric conditions to reveal regions where putative regulators of *AQP* expression might be located. All genes present in these eQTLs were then retrieved and those annotated as transcription factors (TFs) were selected for further analyses. We then performed an enrichment analysis using 45 published gene regulatory networks from different conditions (Zhou et al., 2020, Gomez-Cano et al., 2022). Twenty-four TFs were found in both eQTLs and enrichment analyzes and selected for further wet lab characterization using yeast one-hybrid, dual-luciferase reporter gene, and transactivation assays.

Funding acknowledgement: Fund for Research Training in Industry and Agriculture (FRIA - FNRS), Amaizing (ANR-10-BTBR-0001)

P169

Identification of drought-adaptive QTL underlying variation in root system architecture in Zea mays

(submitted by Kirsten Hein <kirsten.hein@colostate.edu>)
Full Author List: Hein, Kirsten[1]; Woods, Patrick[1]; Mullen, Jack[1]; McKay, John[1]
[1] Department of Agricultural Biology, Colorado State University, Fort Collins, CO 80525

Complex phenotypes are influenced by genetic variation, environmental differences, and genotype-by-environment interactions (G×E). These interacting factors and their relative contributions are therefore of critical importance in understanding complex traits, but at the same time they are challenging to study comprehensively. In part, this challenge stems from the necessary scale: experimental studies of the interaction of genotype and environment must be able to manipulate both factors at a large enough scale to provide the power to detect the relatively small effects of most loci underlying complex traits. Enhanced root systems with deeper architecture are predicted to improve seasonal water-use efficiency, predominantly under drought conditions. To understand the genetic basis for root system architectural variation as it relates to crop production and adaptation to target environments, it is imperative to perform phenotyping under agronomically applicable field conditions. The goal of this study is to evaluate how genes interact with droughted and well-watered environments to create complex root phenotypes in the maize (*Zea may*s L.) system. Quantitative root measurements were collected from small plot field trials of 380 inbred lines of maize across two levels of the environmental factor soil moisture. Genome-wide association (GWAS) identified 85 SNPS significantly associated with root traits, including 12 SNPs that show significant G×E across soil moisture. We then selected biparental recombinant inbred lines that were segregating variation at the genomic regions and gene models identified in the GWAS analysis. Utilizing a computational-based framework proposed by Wen, Pique-Regi, and Luca (2017), we will integrate significant molecular QTLs identified through QTL analysis with the root trait-associated genetic variants to evaluate the enrichment and colocalization of both types of association signals. The result from the integrative analysis has the potential to address more biologically relevant hypotheses by reducing the list of genes within the associated gene set to those genes with the greatest contribution to the overall trait variability using available gene annotation and synteny data.

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

P170

Identification of drought-tolerant hybrids originating from elite parents

(submitted by Dayane Cristina Lima <dclima@wisc.edu>)
Full Author List: Lima, Dayane Cristina[1]; Kaeppler, Shawn M[1]; de Leon, Natalia[1]
[1] Department of Agronomy, University of Wisconsin, Madison, WI 53706, USA

Drought is a major challenge to agricultural production worldwide, and the timing and intensity of this stress can lead to drastic yield losses. In maize (*Zea mays* L.), the effects of drought affect a range of uses, including human and animal consumption and biofuel production. Identifying genotypes tolerant to drought is essential to avoid losses during the growing season and circumvent the environmental and social impact of irrigation. Genotypes representing different heterotic groups display high genetic diversity and are an important source of potential alleles for stress tolerance. This research aims to identify genotypes tolerant to drought and to evaluate grain yield and traits genetically associated with grain yield under drought. We evaluated 1,465 hybrids from three different groups under drought and well-watered conditions from 2018 to 2021 in Graneros, Chile. Group 1 included a set of hybrids derived from the cross of biparental doubled haploid (DH) lines from 12 factorial populations involving seven parents from the non-Stiff Stalk heterotic group crossed with the inbred tester PHT69. Hybrids from group 2 come from DH lines derived from a synthetic population involving parents representing the Stiff Stalk heterotic group and crossed with the inbred tester PHK76. Group 3 is comprised of DH lines derived from PHW65 x PHN11, PHW65 x MoG, and PHW65 x Mo44 crossed with the inbred tester LH195. Of the 1,465 hybrids evaluated in this project, 425 had their performance above the mean in the drought and well-watered treatments considering all years of evaluation. Drought stress started before flowering to cause an asynchrony between anthesis and silking and, consequently, a decline in reproductive success. The parents are elite lines from different sub-heterotic groups and sources of allelic diversity to improve hybrid performance under drought. The main findings of this study will support future breeding efforts for drought tolerance.

Funding acknowledgement: Foundation for Food & Agriculture Research (FFAR) - Crops of the Future

P171 @Mercy92F

Identification of genomic region/s associated with the causal QTL of SHDG trait in Ames panel by GWAS

(submitted by Mercy Fakude <mfakude@iastate.edu>)
Full Author List: Fakude, Mercy[1]; Foster, Tyler L.[1]; Frei, Ursula K.[1]; Lubberstedt, Thomas[1]
[1] Iowa State University; Department of Agronomy; Ames; Iowa, 50011

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. The objective of this genome-wide association study (GWAS) is to identify additional sources of major QTL controlling SHGD.

Funding acknowledgement: United States Department of Agriculture (USDA), US Fulbright Program, National Research Foundation-RISA

P172

Identification of novel QTLs for ear fasciation, kernel row number, ear prolificacy and tillering in maize (*Zea mays* L.)

(submitted by Alberto Tassinari <alberto.tassinari8@unibo.it>)
Full Author List: Tassinari, Alberto[1]; Li, Kai[1]; Giuliani, Silvia[1]; Rosignoli, Serena[1]; Urbany, Claude[2]; Tuberosa, Roberto[1]; Salvi, Silvio[1]
[1] University of Bologna, Bologna, Italy, 40127
[2] KWS SAAT SE & Co. KGaA, Einbeck, Germany

In maize, perturbations of ear meristem proliferation can result in ear fasciation, ie. flattened multiple-tipped ear with disordered kernel arrangement. Ear prolificacy is the development of multiple axillary meristems on the same ear shank or multiple ears per plant. Both ear fasciation and prolificacy may affect grain yield. In the present study we investigate the genetic control of the two traits using two recombinant inbred line populations (B73 × Lo1016 and Lo964 × Lo1016) with Lo1016 and Lo964 as donors of ear fasciation and prolificacy, respectively. The two populations were phenotyped in a multi-year field experiment for ear fasciation-related traits, number of kernel rows (KRN), ear prolificacy and number of tillers. Relatively high heritability values (> 0.5) were detected for all traits. Ear fasciation-related traits were positively correlated with KRN ($0.30 \leq r \leq 0.68$). QTL mapping identified four QTLs for ear fasciation, specifically on chromosomes 1 (two QTLs), 5 and 7. The latter two QTLs overlapped with QTLs for KRN. Two ear fasciation QTLs overlapped with fasciation QTLs previously mapped in other studies and spanned the known ear fasciation genes *compact plant2* and *ramosa1*. Four and five non-overlapping QTLs were mapped for ear prolificacy and tillering, respectively. This study provides novel insights on the genetic control of ear fasciation and describes ear fasciation QTLs that positively affect KRN, one of the most important grain yield component traits. Additionally, we identified novel ear prolificacy and tillering QTLs unexpectedly still segregating in elite maize material.

Funding acknowledgement: KWS SAAT SE & Co. KGaA

P173

Identifying drought tolerance loci through automated root phenotyping and GWAS

(submitted by Grace Sidberry <gcsfgv@umsystem.edu>)
Full Author List: Sidberry, Grace C.[1]; Mitchell, Madison N.[1]; Kick, Daniel R.[2]; Washburn, Jacob D.[2]
[1] Division of Plant Sciences and Technology, University of Missouri, Columbia, Mo 65211
[2] USDA-ARS Plant Genetics Research Unit, USDA-ARS, Columbia, Mo 65211

Drought is one of the most severe abiotic stresses, costing the U.S. over $6 billion annually according to the National Integrated Drought Information system, by reducing the yield of maize and other crops. Finding drought tolerant germplasm and determining the genetic factors influencing it are critical to the development of more resilient crops. A major determinant of crop performance during drought is how well the root system functions to find water deeper in the soil profile. We imaged the primary roots of seedlings from the maize 282/Goodman-Buckler Association Panel in controlled well-watered and water-stressed conditions over the first 48 hours of post-germination growth using a high-throughput phenotyping system and soil-filled transparent plates. Measurements of average root length and root growth rate over time were extracted from these images for use in identifying potentially drought tolerant inbred lines for further study, and to perform a Genome Wide Association Study (GWAS). Examples of Inbred lines found to have similar amounts of primary root growth in both well-watered and water-stressed conditions (potentially drought tolerant) included F44, Mo45, B76, and E22558W. Examples of inbred lines that did not show a drought tolerance primary root phenotype include NC366, CML158Q, and A641. Identified QTL regions and the genes within them will provide valuable targets for further research and use in maize breeding.

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

P174

Image filtering to improve maize tassel detection accuracy using machine learning algorithms

(submitted by Eric Rdoene <eric.rodene@huskers.unl.edu>)
Full Author List: Rodene, Eric[1 2]; Demini Fernando, Gayara[3]; Piyush, Ved[3]; Ge, Yufeng[2 4]; Schnable, James[1 2]; Ghosh, Souparno[3]; Yang, Jinliang[1 2]
[1] Department of Agronomy and Horticulture, University of Nebraska-Lincoln, Lincoln, NE 68583, USA
[2] Center for Plant Science Innovation, University of Nebraska-Lincoln, Lincoln, NE 68583, USA
[3] Department of Statistics, University of Nebraska-Lincoln, Lincoln, NE 68583, USA
[4] Department of Biological Systems Engineering, University of Nebraska-Lincoln, Lincoln, NE 68583, USA

Machine learning-based object detection has become an essential tool in agronomy in recent years, as this provides an automated technique to count germination rates and detect flowering in plants. Unmanned aerial vehicle (UAV)-based imagery has become widely used in collecting time-series agronomic data and generated a large volume of raw data to analyze. Developing automated high-throughput methods of UAV data analysis using machine learning is essential to making efficient analysis possible. Leveraging a published UAV image dataset for a study of 233 inbred lines from the maize diversity panel grown under different nitrogen treatments, we developed machine learning-based techniques for obtaining automated time-series tassel counts at the plot level. In the study, we employed both an object-based, counting-by-detection (CBD) approach and a density-based, counting-by-regression (CBR) approach. Using an image segmentation method that removes most of the pixels not associated with the plant tassels, results showed a dramatic improvement in the accuracy of object-based (CBD) detection, with the cross-validation prediction accuracy (r^2) peaking at 0.7033 on a detector trained with images with a filter threshold of 90. The CBR approach showed the greatest accuracy when using the images filtered at a threshold of 90 as well, with a mean absolute error (MAE) of 8.65. These methods will allow for accurate estimates of flowering-related traits, such as the earliest detected flowering date and the total duration of each plot's flowering period, and will help to guide decisions for future crop improvement.

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

P175

Image-based high-throughput phenotyping and genetic analysis of plant architecture in maize

(submitted by Yawei Li <yaweil@iastate.edu>)
Full Author List: Li, Yawei[1]; Qiao, Yang[1]; Jubery, Zaki[1]; Liu, Xuan[1]; Qiu, Yumou[1]; Tang, Lie[1]; Ganapathysubramanian, Baskar[1]; Schnable, Patrick S[1]
[1] Iowa state university

Breeding maize for increased grain yield has resulted in substantial changes in plant architecture, including more upright leaves that decrease mutual shading and increased tolerance to higher plant densities. Various imaging approaches have been developed for high-throughput phenotyping of whole plants. We applied three phenotyping pipelines to extract unidimensional and multidimensional traits from field-grown plants imaged either non-destructively in the field or destructively sampled and imaged indoors. **1. Time-lapse imaging *in the field*.** Hundreds of cameras were mounted on poles in the field to collect a series of time-lapse images over time with a focus on plant height and leaf angles. Images were taken every 15 mins from 8 am to 8 pm throughout the growing season. Our current pipeline can accurately extract plant heights from images to construct dynamic growth curves. However, this pipeline still limits our ability to detect and segment leaf traits across whole plants. **2. Phenobot imaging of plants *in the field*.** We also used a robotic phenotyping system, "PhenoBot 3.0" to capture and reconstruct the 3-D structures of field-grown plants. More than 26,000 plants can be phenotyped in 4 hours, greatly increasing data collection efficiency. Larger numbers of samples per genotype can help the control for plant-to-plant variation in leaf angles. Thus, we can more accurately identify general patterns of differences of leaf angle across plants within genotypes. **3. High-resolution indoor imaging of destructively sampled field-grown plants.** Field-grown plants were harvested and transferred to an indoor imaging station outfitted with high-resolution cameras, a uniform background, and optimal lighting. This allows detailed leaf traits from each individual plant to be captured. Hence, using the approach it will be possible to extract multiple leaf traits, including leaf angles. In conclusion, although the three phenotyping pipelines have different strengths and weakness, collectively they can be used to accurately extract diverse plant architecture traits from images. As such, they will support the identification of candidate genes associated with variation in plant architecture.

Funding acknowledgement: United States Department of Agriculture (USDA)

P176

Improving the use of plant genetic resources to sustain breeding programs efficiency

(submitted by Alain Charcosset <alain.charcosset@inrae.fr>)
Full Author List: Sanchez, Dimitri[1]; Ben-Sadoun, Sarah[1]; Mary-Huard, Tristan[1 2]; Allier, Antoine[3]; Laurence, Moreau[1]; Charcosset, Alain[1]
[1] Université Paris-Saclay, INRAE, CNRS, AgroParisTech, UMR Génétique Quantitative et Evolution Le Moulon, 12 route 128, Gif-sur-Yvette, 91190, France
[2] MIA, INRAE, AgroParisTech, Université Paris-Saclay, Paris, 75005, France
[3] Syngenta, 12 Chemin de l'Hobit, Saint-Sauveur, 31790, France

Breeding is accompanied by a loss in genetic diversity, which hinders sustainable genetic gain. Methodologies based on molecular marker information have been developed to manage diversity and proved effective in increasing long-term genetic gain. However, with realistic plant breeding population sizes, diversity depletion in closed programs appears ineluctable, calling for the introduction of relevant diversity donors. Although maintained with significant efforts, genetic resource collections remainunderutilized, due to large performance gap with elite germplasm. Bridging populations created by crossing genetic resources to elite lines prior to introduction into elite programs can manage this gap efficiently. To improve this strategy, we explored with simulations different genomic prediction and genetic diversity management options for a global program involving a bridging and an elite component. We analyzed the dynamics of quantitative trait loci fixation and followed the fate of allele donors after their introduction into the breeding program. Allocating 25% of total experimental resources to create a bridging component appears highly beneficial. We showed that potential diversity donors should be selected based on their phenotype rather than genomic predictions calibrated with the ongoing breeding program. We recommend to incorporate improved donors into elite using a global calibration of the genomic prediction model and optimal cross selection maintaining a constant diversity. These approaches use efficiently genetic resources to sustain genetic gain and maintain neutral diversity, improving the flexibility to address future breeding objectives.

Funding acknowledgement: INRAE, Promaïs (KWS, Lidea, Limagrain, MassSeeds, RAGT)

P177

Increased stem sink strength and sugar accumulation using male sterility in *Sorghum bicolor*

(submitted by Liz Dominguez <lizethd2@illinois.edu>)
Full Author List: Dominguez, Liz[1]; Studer, Anthony J[1]
[1] Department of Crop Sciences, University of Illinois Urbana-Champaign, Urbana, IL 61801, USA

Emerging new technologies can now use fermentable sugars for the production of sustainable aviation fuels (SAF). With this, comes the demand for diverse, high yielding input sugar sources. Sorghum has the potential to supply sugars to the SAF market while also having the added advantages of stress tolerance and the ability to grow on marginal land. However, in order for sorghum to be a viable bioenergy crop, improvements need to be made to increase the quantity of sugar accumulated in the stem. Our work investigates the use of cytoplasmic male sterile (CMS) lines to increase sugar accumulation in the stem by removing the panicle as a sink tissue. To test the extent to which resources could be redirected to the stem, four CMS lines were grown alongside their fertile counterpart in replicated field trials in 2020 and 2022. Bags were placed over the panicles of CMS lines to prevent seed production and fertile lines were open pollinated. By inhibiting seed set we were able to test each line's ability to increase stem sink strength. Our data shows that preventing seed set can alter stem sink strength; however, the effect was genotype dependent. Only some lines showed a significant difference in sugar production, indicating an underlying difference in the source-sink programming. In addition, increases could be seen in either juice volume, and/or sugar concentration in the stem. These results suggests that multiple mechanisms play a role in the concentration of sugars and accumulation of juice to the stem. Dissecting these mechanisms will be the focus of our future work to improve our understanding of source-sink relationships and allow for the development of higher yielding crops.

Funding acknowledgement: Department of Energy (DOE)

P178

Investigating heterosis for resistance to three pathogens

(submitted by Asher Hudson <ahudson3@ncsu.edu>)
Full Author List: Hudson, Asher I[1]; Wagner, Maggie R[2]; Balint-Kurti, Peter J[1 3]
[1] Department of Entomology and Plant Pathology, North Carolina State University, Raleigh, NC, USA 27695
[2] Department of Ecology and Evolutionary Biology, Kansas Biological Survey and Center for Ecological Research, University of Kansas, Lawrence, KS, USA 66045
[3] USDA-ARS Plant Science Research Unit, Raleigh, NC, USA 27695

Most maize agriculture makes use of heterosis, yet the mechanisms causing it are still under debate. Increased yield in hybrids is often hypothesized to be the result of nonadditive gene action which likely acts on underlying traits. Loci underlying disease resistance often display nonadditive behavior, yet research on heterosis for disease resistance has had inconsistent results. We phenotyped the intermated B73 x Mo17 intercross (IBM) population, as well as backcrosses of the IBM lines to each parent, for resistance to three pathogens; *Cercospora zeae-maydis*, *Exserohilum turcicum* and *Cochliobolus heterostrophus*. We then performed a quantitative trait locus (QTL) analysis separately in the IBM lines and backcrosses of these lines to each parent. This enabled us to investigate whether QTL showed behavior consistent with additive, dominant, recessive, or overdominant gene action. We found little evidence for heterosis for disease resistance to *E. turcicum* or *C. heterostrophus* in our experiment and some evidence for heterosis for resistance to *C. zeae-maydis*. This may be explained by the fact that B73 and Mo17 each carry both dominant and recessive resistance (or dominant susceptibility) alleles at different loci, resulting in a hybrid phenotype similar to the mid-parent value. We also found no evidence for overdominance even when relaxing significance thresholds. Our results show that despite nonadditive gene action being common for disease resistance loci, the distribution of dominant and recessive resistance alleles may prevent the expression of heterosis.

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

P179

Investigating leaf-water relations and stomatal control in *Zea mays* using the mutant *slac1-2*

(submitted by Robert Twohey III <twohey2@illinois.edu>)
Full Author List: Twohey III, Robert J[1]; Li, Catherine H[1]; Hanneman, Marjorie[2]; Wickes-Do, Liam[2]; Gore, Michael A[2]; Moose, Stephen P[1]; Studer, Anthony J[1]
[1] Department of Crop Sciences, University of Illinois, Urbana, IL 61801, USA
[2] Plant Breeding and Genetics Section, School of Integrative Plant Science, Cornell University, Ithaca, NY 14853, USA

Stomata control CO_2 and water vapor movement across the leaf surface by altering their aperture via guard cells. SLAC1 is a guard cell-specific anion channel controlling stomatal movement. Our lab characterized a null mutant *slac1-2* in *Zea mays* that is unable to close its stomata in response to multiple environmental stimuli. Here we present the use of *slac1-2* to investigate the level of stomatal control as *Z. mays* responds to increasing vapor pressure deficit (VPD), how stomata affect nitrogen uptake, and determine if stomata limit photosynthetic efficiency in the C_4 crop *Z. mays*. As atmospheric VPD increases, there is an increased demand for water from the surrounding air. Past studies have identified VPD breakpoints where the linear transpirational response to increasing VPD changes in slope. It has been hypothesized that breakpoints are controlled by hydraulic conductance. We performed VPD curves on *slac1-2* to determine if a plant without stomatal control shows a VPD breakpoint due to alternate intracellular regulators of transpiration. Breakpoints were not observed in *slac1-2* hybrids, indicating that stomata are the main regulators during VPD response. Since the *slac1-2* mutant had previously only been evaluated in an inbred genetic background, single-cross hybrids homozygous for the *slac1-2* mutation were produced. The *slac1-2* hybrids and wild type comparisons were planted in three locations during the 2022 field season. Midday gas exchange measurements and yield data were collected from the plots to determine if a constant open stomata phenotype removes a possible CO_2 photosynthetic limitation in a hybrid background. Stomatal conductance was significantly higher in the *slac1-2* hybrid however, this did not result in significantly higher yields at any of the field sites. Plant nitrogen content was also measured in the hybrids, and results support *slac1-2* having an impact on nitrogen accumulation and partitioning.

Gene / Gene Models described: *slac1-2*; Zm00001eb073420

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

P180

Key developmental windows and environmental parameters that influence cuticular wax composition on maize silks

(submitted by Matthew Wendt <wendtmat@iastate.edu>)
Full Author List: Wendt, Matthew M.[1]; Hattery, Travis J.[1]; Chen, Keting[1]; Granados-Nava, Karen[1 2]; Schroeder, Elly[1 3]; Myers, Bryn[1 2]; Moore, Riley[1 4]; Loneman, Derek[1]; Claussen, Reid[1]; Gilbert, Amanda[1]; Garfin, Jacob[5]; Bjerklie, Dane[5]; Lauter, Nick[6 7]; Hirsch, Candice N.[5]; Yandeau-Nelson, Marna D.[1]
[1] Department of Genetics, Development, and Cell Biology, Iowa State University; Ames, Iowa 50011, USA
[2] Undergraduate Genetics Major, College of Liberal Arts and Science, Iowa State University; Ames, Iowa 50011, USA
[3] Undergraduate Kinesiology Major, College of Human Sciences, Iowa State University; Ames, Iowa 50011, USA
[4] Undergraduate Biology Major, College of Liberal Arts and Sciences, Iowa State University; Ames, Iowa 50011, USA
[5] Department of Agronomy and Plant Genetics, University of Minnesota; Minneapolis, Minnesota 55455, USA
[6] Department of Plant Pathology and Microbiology, Iowa State University; Ames, Iowa 50011, USA
[7] USDA-ARS Corn Insects and Crop Genetics Research Unit, Iowa State University; Ames, Iowa 50011, USA

Desiccation of maize silks hinders fertilization; thus, silk drought tolerance is a potential breeding goal to maintain or enhance maize productivity in increasingly dry environments. Maize silks, and other aerial organs, are coated with a hydrophobic cuticle that limits water loss and protects against other abiotic and biotic stresses. Prior work has found silk cuticular wax accumulation increases dramatically in growing seasons that experience low precipitation. Moreover, cuticular wax composition can vary among genotypes and accumulate to higher concentrations in portions of the silks that are emerged from encasing husk leaves into the external environment. While genotype-by-environment effects have been implicated in cuticular wax accumulation, a true multivariate analysis of cuticular wax phenotypes is still needed. To parse the effects of genotype and environment on cuticular waxes, 468 Wisconsin Diversity Panel inbreds were grown in both Iowa and Minnesota in 2016 to 2017, providing silk cuticular wax data for 45 metabolites across three environments, with high resolution weather data at each site. A similar experiment was performed in 2020 wherein 11 strategically selected inbreds from this panel were grown across 6 planting dates in both Iowa and Minnesota. Linear modeling and canonical correlation analysis were used to find putative associations between silk cuticular waxes and weather throughout plant development. Low precipitation and high solar radiation immediately prior to silking were correlated with increased cuticular wax accumulation, though intensity of solar radiation weeks prior to silking may also influence the silk cuticle. This analysis provides a foundation for controlled environment studies that assess the impact of specific weather parameters at specific stages in plant development on the silk cuticle. Ultimately, this research will lead to a better understanding of abiotic factors that impact cuticle composition and whether these modified cuticles protect against specific stresses.

Funding acknowledgement: National Science Foundation (NSF)

P181

Large-scale multi-environment genomic prediction for maize hybrid selection

(submitted by Haixiao Hu <hxhu@ucdavis.edu>)
Full Author List: Hu, Haixiao[1]; Runcie, Daniel[1]
[1] Department of Plant Sciences, University of California, Davis, CA 95616, USA

In maize breeding, multi-environment trials are important for assessing the performance of hybrids across environments and identifying well-adapted genotypes for a specific region. However, plant breeding programs are limited in the number of genotypes and environments that can be assessed due to limited budgets. It is never possible to test all possible hybrid crosses in all possible environments (especially future environments). Multi-trait genomic prediction models can help plant breeders leverage multi-environment trials to select locally adapted genotypes by learning the genotype-performance relationship within trials and genetic correlations among trials. The recently completed Genomes to Fields (G2F) Genotype by Environment Prediction Competition was initiated to assess how different modeling approaches can predict maize yields across North America. However, this competition focused on the most challenging type of genotype-by-environment prediction: predicting the performances of newly created genotypes in future environments, and explicit multi-environment models were not highly successful. Here we explore the question of whether multi-environment data can be useful in more limited contexts: predicting the relative performances of 1) previously seen genotypes in new environments, and 2) newly created genotypes in previously seen environments. Both are useful for breeders to save costs either by reducing the number of test environments or by reducing the number of genotypes to be tested in each environment. We will use the G2F data to assess the advantage of multi-trait genomic predictions in each case using our recently developed statistical model MegaLMM which is able to efficiently fit multi-trait genomic predictions for hundreds of trials simultaneously. These results will expand our understanding of genomic prediction strategies for testing maize hybrids in multi-environment trials and provide maize breeders the means to identify the best hybrids with reduced costs.

Funding acknowledgement: United States Department of Agriculture (USDA)

P182 @rafael_coletta

Linking genetic and environmental factors through marker effect networks to understand trait plasticity

(submitted by Rafael Della Coletta <della028@umn.edu>)
Full Author List: Della Coletta, Rafael[1]; Liese, Sharon E.[2]; Fernandes, Samuel B.[3]; Mikel, Mark A.[2 4]; Bohn, Martin O.[2]; Lipka, Alexander E.[2]; Hirsch, Candice N.[1]
[1] Department of Agronomy and Plant Genetics, University of Minnesota, St. Paul, MN 55108, USA
[2] Department of Crop Sciences, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA
[3] Department of Crop, Soil, and Environmental Sciences, University of Arkansas, Fayetteville, AR 72701, USA
[4] Roy J. Carver Biotechnology Center, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA

Understanding how plants adapt to specific environmental changes and identifying genetic markers associated with phenotypic plasticity can help breeders develop plant varieties adapted to a rapidly changing climate. Here, we propose the use of marker effect networks as a novel method to identify markers associated with environmental adaptability. These marker effect networks are built by adapting commonly used software for building gene co-expression networks with marker effects across growth environments as the input data into the networks. To demonstrate the utility of these networks, we built networks from the marker effects of ~10,000 non-redundant markers from 400 maize hybrids across nine environments. We demonstrate that networks can be generated using this approach, and that the markers that are co-varying are rarely in linkage disequilibrium, thus representing higher biological relevance. Multiple covarying marker modules associated with different weather factors throughout the growing season were identified within the marker effect networks. Finally, a factorial test of analysis parameters demonstrated marker effect networks are relatively robust to these options, with high overlap in modules associated with the same weather factors across analysis parameters. This novel application of network analysis provides unique insights into phenotypic plasticity, and specific environmental factors that modulate the genome.

Funding acknowledgement: United States Department of Agriculture (USDA)

P183

Low-cost phenotyping of seedling root growth angle shows genotypic variation and plastic response to parental nitrogen input

(submitted by Ella Townsend <ellatown@iastate.edu>)
Full Author List: Townsend, Ella G.[1]; Klein, Stephanie P.[1]; Anderson, Sarah N.[1]
[1] Department of Genetics, Development & Cell Biology, Iowa State University, Ames, IA, 50011

Since large portions of the world's agriculture is grown on land with low fertility, developing varieties that efficiently acquire nutrients from the soil is critical for maize production. Past research has shown that maize with a steep root growth angle (RGA) is better able to acquire nitrogen (N), especially in environments where N availability is limited. RGA is also plastic in response to stress. We are interested in better understanding the diversity of root plastic responses to N-stress and if the plasticity response can be carried into the next generation. We developed a low-cost method to screen RGA in 12-day-old seedlings and used this method to measure seedling roots whose parents were grown in either a high or low N field environment. Using a scanner, we generated a root architecture image library of 12-day-old maize seedlings that were grown vertically in germination paper in a growth chamber and used ImageJ to measure RGA in the first nodal root. Within the 16 genotypes we evaluated, we observed variation in RGA and variable plastic responses to the parental N input. These findings indicate that not only have we devised a system that enables us to quickly screen large germplasm collections for RGA diversity, but also that environmental stress may induce phenotypic changes in young seedlings. Moving forward, we are planning on adapting our phenotyping workflow to integrate other software programs, like RhizoVision, to expand the number of root traits that can be quantified.

Funding acknowledgement: United States Department of Agriculture (USDA)

P184

Maize breeding impacts on sustainability

(submitted by Sotirios Archontoulis <sarchont@iastate.edu>)
Full Author List: Archontoulis, Sotirios[1]; Trifunovic, Slobodan[2]; Eddy, Doug[2]; Vyn, Tony[3]; Topp, Chris[4]; Lamkey, Kendall[1]; Ruiz, Alejo[1]; King, Kyle[1]; Olmedo Pico, Lia[3]; Thies, August[4]
[1] Iowa State University
[2] Bayer Crop Science
[3] Purdue University
[4] Danforth Center

Maize breeding has contributed to increased crop productivity over the years, however, the impact of maize breeding on sustainability remains unknown. Here we present new data from a comprehensive experiment carried out in the US Corn Belt to address this knowledge gap. A set of historical maize hybrids (released from 1980 to 2020) were grown across 100 environments (site-years) having different plant densities, N-fertilizer, fungicide, and irrigation treatments. Results indicated that maize breeding has increased grain yields per unit land area and per plant while simultaneously increasing nitrogen-water-radiation use efficiencies. Through process-based simulation modeling, we found that these changes resulted in lower N_2O emissions and N leaching losses. We conclude that maize breeding has significantly contributed to both increased crop productivity and sustainability over the last 40 years.

Funding acknowledgement: FFAR and Bayer Crop Science

P185

Mapping maize brace root traits associated with nitrogen-fixing bacterial symbiosis

(submitted by Daniel Laspisa <Daniel.Laspisa@uga.edu>)
Full Author List: Laspisa, Daniel[1]; Phillips, Courtney[1]; Griffis, Holly[1]; Wallace, Jason[1]
[1] University of Georgia, Crop and Soil Sciences, Institute of Plant Breeding, Genetics and Genomics, CAGT, 111 Riverbend Rd, Athens, GA 30602

Mucilage is the carbohydrate-rich gelatinous substance produced by maize on underground and nodal root tips. In the rhizosphere it lubricates the root as it grows through the soil and improves water/nutrient uptake. An indigenous variety of maize grown in the Sierra Mixe region of Oaxaca, Mexico was found to produce extensive nodal roots. The nodal roots produce abundant mucilage that harbors diazotrophic bacteria allowing it to thrive in nitrogen-depleted soils. Similar microbial interactions are well characterized in legumes but little is known about such interactions in cereals. Here we evaluate a BC1S2 population of accession Oaxaca184 (PI 629238) backcrossed to PH207 and genotyped using rAMP-seq. We estimated the heritability of various traits related to the nitrogen-fixation symbiosis (nodal root number, nodal root size, number of roots, etc.), their relationship to agronomic traits like flowering time and plant height, and mapped QTL associated with each of these traits. The identification of regions controlling root and mucilage traits will provide a deeper understanding of the trait and how to transfer it to other materials.

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

P186

Metabolic pathway associations of maize stalk lodging resistance

(submitted by Norbert Bokros <nbo245@uky.edu>)
Full Author List: Bokros, Norbert T[1]; Verges, Virginia[1]; Kunduru, Bharath[3]; Stubbs, Christopher J[2]; Tabaracci, Kaitlin[3]; Cook, Douglas D.[5]; Robertson, Daniel J[3]; McMahan, Christopher S.[6]; Sekhon, Rajandeep S.[4]; DeBolt, Seth[1]
[1] Department of Horticulture, University of Kentucky, Lexington, KY, USA 40546
[2] School of Computer Sciences and Engineering, Fairleigh Dickinson University, Teaneck, NJ, USA 07666
[3] Department of Mechanical Engineering, University of Idaho, Moscow, ID, USA 83844
[4] Department of Genetics and Biochemistry, Clemson University, Clemson, SC, USA 29634
[5] Department of Mechanical Engineering, Brigham Young University, UT, USA 84602
[6] School of Mathematics and Statistical sciences, Clemson University, Clemson, SC, USA 29634

Stalk lodging in maize occurs when aboveground structural biomass critically fails at or below the primary ear-bearing node, reducing global grain yields by 5-20% each year. Several isolated environmental, architectural, mechanical, and genetic predeterminants of stalk lodging susceptibility are under study, however the problem of stalk lodging in grains persists. Rather than isolating a single feature of stalk lodging susceptibility, this study seeks a unified approach to identify underlying metabolic pathways significantly associated and shared amongst multiple predeterminant phenotypes associated with stalk lodging resistance. To this end, 7 maize architectural and mechanical phenotypes were collected across two location and two years within 264 maize inbred genotypes. GWAS, utilizing either 164k or 17.7 million SNPs, were used to highlight regions of the maize genome significantly associated with phenotypic determinants of stalk lodging resistance. Metabolic pathway analysis – utilizing PAST – was then performed as a compliment to GWAS to identify and highlight the most biologically relevant genes and pathways involved in phenotype improvement. The results of this study should help uncover the genetic underpinnings of stalk lodging resistance by identifying and associating the cumulative effect of multiple genes within linkage blocks with known metabolic pathways.

Funding acknowledgement: National Science Foundation (NSF)

P187 @DrAngelaKent

Mining ancient genomes for crops of the future

(submitted by Angela Kent <akent@illinois.edu>)
Full Author List: Kent, Angela D.[1]; Favela, Alonso[2]; Bohn, Martin[3]; Woodward, Logan[3]; Ghotbi, Mitra[1]; Sible, Connor[3]; Below, Frederick[3]
[1] Department of Natural Resources & Environmental Sciences, University of Illinois at Urbana-Champaign
[2] Department of Ecology and Evolutionary Biology, University of California-Irvine
[3] Department of Crop Sciences, University of Illinois at Urbana-Champaign

Plant-microbe interactions in the rhizosphere govern the 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 large 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 germplasm selected under different levels of fertilization. Ancestral genotypes inhibited nitrification (BNI), a capability that may contribute to N sustainability. Maize-teosinte NILs with introgressions for BNI were identified and grown in a field experiment to examine the effect on N sustainability. We further paired this trait with N-fixing inoculants to determine if nitrification inhibition can improve N acquisition in maize. Our results link the host-associated microbiome and ecosystem function, and demonstrate the genetic capacity to optimize the recruitment of N-cycling functional groups and improve crop sustainability. Understanding this relationship will allow breeders and ecosystem scientists to develop crop cultivars that interact with the nitrogen cycle in predictable beneficial ways to improve the efficiency and sustainability of agriculture, while protecting environmental quality.

Funding acknowledgement: United States Department of Agriculture (USDA), Illinois Nutrient Research and Education Council

P188

Monitoring nitrogen-responsive phenotypes in corn with the TerraSentia Rover

(submitted by Grace Nystrom <gln2@illinois.edu>)
Full Author List: Nystrom, Grace L.[1]; McGuire, Michael[2]; Kumar Uppalapati, Naveen[3]; Aref, Thomas[2]; Li, Catherine H.[1]; Chowdhary, Girish[2 3 4]; Moose, Stephen P.[1]
[1] Department of Crop Sciences, University of Illinois Urbana-Champaign, Urbana, IL 61801, USA
[2] EarthSense Co., Champaign, IL 61820, USA
[3] Department of Agricultural and Biological Engineering, University of Illinois Urbana-Champaign, Urbana, IL 61801, USA
[4] Department of Computer Science, University of Illinois Urbana-Champaign, Urbana, IL 61801, USA

Measuring maize phenotypes that respond to nitrogen supply is challenging due to spatial variability in soil N and labor-intensive methods for measuring growth and partitioning of plant N. The TerraSentia rover, developed by the autonomous robotics company EarthSense, can be programmed to move through field rows and scan plant spectral characteristics using multiple mounted sensors and intuitive on-board algorithms. These automated imaging capabilities offer new opportunities to measure nitrogen-responsive phenotypes inside the canopy. We deployed the TerraSentia rover multiple times during the 2021 and 2022 seasons to collect data from 500- plus plots in an experimental design that included different N fertilizer treatments, inbreds and hybrids, and genetic variants known to impact nitrogen utilization traits. Ground-truth data for each trait was also collected manually from a subset of the plots. We found that the rover was able to accurately estimate total plant height (r^2 >0.94) and ear height (r^2 >0.87), and for both traits could identify phenotypic changes associated with genetic variants. The rover detected decreases in plant height due to N-deficiency, but ear height was not highly responsive to N whether measured manually or by rover. Rover algorithms also estimated leaf area index and stem width, initial predictions of stem width were inaccurate, analyses of leaf area index are in progress. Overall, the TerraSentia rover shows significant promise for automated proximal phenotyping of nitrogen utilization traits in maize field experiments.

Funding acknowledgement: National Science Foundation (NSF)

P189

Multi-environmental RNAseq reveals the extent of Genotype-by-Environment interactions for gene expression.

(submitted by Ty Thomas <tsthoma2@ncsu.edu>)
Full Author List: Thomas, Ty S[1]; Rogers, Anna[10]; Buckler, Edward S[2 3 4]; Kaeppler, Shawn[5]; de Leon, Natala[5]; Flint-Garcia, Sherry[6 7]; Hirsch, Candice[8]; Springer, Nathan[10]; Romay, Cinta[4]; Holland, James[1 9]; Gage, Joseph[1]
[1] Department of Crop and Soil Sciences, North Carolina State University, Raleigh, NC 27695
[2] Agricultural Research Service, US Department of Agriculture, Ithaca, NY 14853
[3] Plant Breeding and Genetics Section, School of Integrative Plant Science, Cornell University, Ithaca, NY 14853
[4] Institute for Genomic Diversity, Cornell University, Ithaca, NY 14853
[5] Department of Agronomy, University of Wisconsin - Madison, Madison WI 53706
[6] Agricultural Research Service, US Department of Agriculture, Columbia, MO 65211
[7] Division of Plant Science and Technology, University of Missouri, Columbia, MO 65211
[8] Department of Agronomy and Plant Genetics, University of Minnesota, St Paul MN 55108
[9] Agricultural Research Service, US Department of Agriculture, Raleigh, NC 27695
[10] Bayer Crop Science, Chesterfield, MO 63017

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 the amplitude of 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 are able to quantify gene expression and create statistical models to account for genetic variation, environmental variation, and GxE variation on a single gene basis. Models estimate the median of expression variation for genotype, environment, and GxE to be 3.4%, 25.7%, and 6.3% respectively. We found 10, 951,and 43 genes where over 50% of variation in expression is controlled by genotype, environment, and GxE, respectively.We plan to use the Practical Haplotype Graph (PHG), to map reads against a pangenome and assign them to haplotype groups, giving more statistical power and utilizing the breadth of information provided by the pangenome. These models bring novel understanding of individual gene expression as it pertains to GxE in maize and can be used to predict the level of expression a gene will have in a given environment. 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.

P190

Multi-year, holistic phenotyping reveals genetic diversity in nitrogen related traits

(submitted by Collin Luebbert <cluebbert@danforthcenter.org>)
Full Author List: Luebbert, Collin V.[1]; Rajurkar, Ashish B.[2]; Leakey, Andrew D.B.[2]; SINC, Consortium[1]
[1] Donald Danforth Plant Science Center, Saint Louis, MO 63132, USA
[2] Department of Plant Biology, Institute for Genomic Biology, University of Illinois, Urbana, IL 61801, USA

Nitrogen inputs are an important consideration in terms of greenhouse gas emissions as well as environmental impacts from pollution. In order to maintain yields while decreasing these inputs we have to consider ways that we can breed maize that is more efficient in its use of nitrogen. Over multiple growing seasons, we have conducted a number of experiments that seek to study genetic diversity of many nitrogen related phenotypes in two different populations. We grew the Buckler-Goodman diversity panel and a set of synthetic maize lines derived from nested-associated mapping population parents and synthetic teosinte lines (Zea synthetics). We heavily phenotyped these lines over two growing seasons in nitrogen limited conditions to measure root traits, elemental concentrations, nitrogen content and other agronomically important traits. The Buckler-Goodman panel was used in a genome-wide association study to identify associated loci with all traits measured. Zea synthetics are currently being genotyped with the goal of performing the same analysis. To obtain a more holistic understanding of the factors influencing nitrogen efficiency, a subset of these Zea synthetic lines was chosen based on interesting root and yield characteristics and grown up in two additional seasons. In addition to agronomic, elemental and root traits we collected microbiome and metabolome samples. Overall, we have identified genetic diversity in nitrogen related traits which can serve as a foundation for identifying causal loci in the maize genome.

Funding acknowledgement: National Science Foundation (NSF)

P191 @_dianamarcela__

Optimizing genomic prediction for the germplasm enhancement of maize project

(submitted by Diana Marcela Escamilla Sanchez <descamil@iastate.edu>)
Full Author List: Escamilla, Diana M.[1]; Li, Dongdong[1]; Kappelmann, Kiara L.[1]; Li, Xianran[2]; Peters, David W.[3]; Vanous, Adam E.[3]; Yu, Jianming[1]
[1] Department of Agronomy, Iowa State University, Ames, IA, USA
[2] USDA ARS, Wheat Health, Genetics, and Quality Research Unit, Pullman, WA, USA
[3] USDA ARS, North Central Regional Plant Introduction Station, Ames, IA, USA

Landraces from tropical regions are valuable genetic resources for public and private breeding programs. Their integration and maintenance into elite germplasm are critical to safeguarding maize from future threats. With the advances in genomic technologies, low-cost genotyping, and genomic selection, the efficient mining of diverse crop germplasm is now possible. The Germplasm Enhancement of Maize (GEM) project is an ongoing initiative of public and private sector breeders to incorporate genetic diversity from exotic lines into maize breeding programs. By crossing exotic parents with temperate elite lines, the GEM project produces lines containing 25-50% exotic germplasm that can readily be incorporated into elite temperate breeding programs. The initial attempt to integrate genomic selection (GS) into the GEM project showed that models developed within heterotic groups and breeding programs could achieve prediction accuracies of 0.36 to 0.75 based on topcross yield and genomic data. Improved GS models and optimized training populations are therefore necessary for improving prediction accuracy and connectivity between breeding programs. A large collection of maize lines containing 50% exotic germplasm, developed by crossing tropical landraces from eight south and central American countries with an elite proprietary tester, has recently been donated to the GEM project by Bayer. This study aims to apply design thinking to optimize the process to study this collection utilizing recent innovations in high-throughput sequencing, training set design, and genome prediction for diverse germplasms. Lines will be genotyped using genotyping by sequencing and phenotyped for topcross yield and other traits of interest. The steps we will undertake include optimal training population design, cross-validation, empirical validation experiments, model updating, and further testing. This research will help to accelerate the GEM lines release process.

Funding acknowledgement: USDA-ARS Germplasm Enhancement of Maize, USDA-NIFA AFRI (2021-67013-33833)

P192 @lindachidao

Pericarp thickness in sweet corn inbreds and hybrids

(submitted by Linda Dao <ldao@ufl.edu>)
Full Author List: Dao, Linda C.[1]; Gustin, Jeff L.[2]; Resende, Marcio F. R.[1]; Hannah, L. Curt[1]; Settles, A. Mark[1 3]
[1] Horticultural Sciences Department, University of Florida, Gainesville, FL 32611
[2] Maize Genetics Cooperation - Stock Center, USDA-ARS, Urbana, IL 61801
[3] Present address: NASA Ames Research Center, Moffett Field, CA 94035

The pericarp, which is the maternal tissue surrounding the kernel, provides strength and resistance against moisture, insects, and fungal pathogens. Its thickness impacts the texture and quality of the kernel. While thinner lines are preferred in taste panels, thicker lines may prevent damage to the seed at harvest and planting. The thickness of the pericarp is controlled by multiple genes and demonstrates partial dominance of thin pericarp. In addition to its high narrow sense heritability, variation is controlled by dominant and epistatic effects. Previously, mature pericarp thickness has been predicted using near-infrared spectroscopy (NIR). This project aims to investigate the relationship between thickness at 21 days after pollination (DAP) and maturity. We measured the thickness of excised pericarp in 120 sweet corn inbred lines at eating stage (21 DAP) and maturity with a pressure micrometer. Abgerminal, germinal, and crown measurements were taken. Hybrids were then made with four thick lines and two thin lines crossed to an intermediate tester. We show there is a strong correlation between eating stage and mature stage thickness, which suggests we can apply non-destructive methods like NIR prediction to select agronomically important lines before planting. Furthermore, there is no relationship between pericarp thickness and germination. When making hybrids, crossing thick lines with thinner lines can decrease the thickness. These results demonstrate the possibility of rapidly selecting and breeding towards thinner lines without sacrificing seed vigor.

Funding acknowledgement: United States Department of Agriculture (USDA)

P193 @coop0409

Phenomic and genomic temporal analysis of maize canopy cover

(submitted by Julian Cooper <coop0409@umn.edu>)
Full Author List: Cooper, Julian S.[1]; Sweet, Dorothy D.[1 2]; Tirado, Sara B.[2 3]; Springer, Nathan M.[3]; Hirsch, Candice N.[2]; Hirsch, Cory D.[1]
[1] Department of Plant Pathology, University of Minnesota, Saint Paul, MN 55108
[2] Department of Agronomy and Plant Genetics, University of Minnesota, Saint Paul, MN 55108
[3] Department of Plant and Microbial Biology, University of Minnesota, Saint Paul, MN 55108

Canopy cover is an important trait associated with biomass accumulation, photosynthesis, harvest index, and weed suppression. The quantitative, continuous nature of canopy cover makes repeated measurements beneficial for understanding how time, environment, and genetic factors influence this trait. Utilizing high-throughput remote sensing technology, aerial RGB images of the maize Wisconsin Diversity Panel were captured throughout the 2018-2021 growing seasons at the University of Minnesota St. Paul campus field plots. Field image orthomosaics were masked using k-means clustering to calculate percent canopy cover as the ratio of plant pixels per plot. Using a loess local regression to predict percent plot canopy coverage at 50 growing degree days (GDD) intervals from 300-1650 GDD in each growth environment, we observed that canopy development followed a logistic growth curve. Genetic, environmental, and the GxE interaction all contributed substantial percent variance explained, with environment explaining the most variation during the adult vegetative growth stage from approximately 500-1100 GDD. Additive main effect and multiplicative interaction and Finley-Wilkinson regression analysis demonstrated that canopy cover was relatively stable in these environments, however certain genotypes displayed increased phenotypic plasticity during the adult vegetative growth stage. A temporal genome wide association study identified distinct and shared marker trait associations across time, between environments, and amongst trait and stability metrics with varied temporal durability depending on the trait-environment combination. This study explored the variation in maize canopy phenotype development and associated genomic regions in response to temporal and environmental factors and highlights the necessity of high-resolution, longitudinal analyses for dissecting complex traits.

Funding acknowledgement: National Science Foundation (NSF), Bayer Crop Science/University of Minnesota Multifunctional Agriculture and Food System Initiative Graduate Student Fellowship, Minnesota Agricultural Experimental Station Hatch project MIN-22-086, NSF, Minnesota Corn Growers Association

P194

Phenotypic Plasticity: A broad framework to reinstate the environmental dimension for GWAS and genomic selection

(submitted by Jianming Yu <jmyu@iastate.edu>)
Full Author List: Yu, Jianming[1]; Guo, Tingting[2]; Mu, Qi[1]; Tibbs-Cortes, Laura E.[1 3]; Wei, Jialu[1]; Alladassi, Boris M.E.[1]; Vanous, Adam E.[4]; Li, Xianran[3]
[1] Department of Agronomy, Iowa State University, Ames, IA, USA 50011
[2] National Key Laboratory of Crop Genetic Improvement, Huazhong Agricultural University, Wuhan, China 430070
[3] USDA-ARS, Wheat Health, Genetics, and Quality Research Unit, Pullman, WA, USA 99164
[4] USDA ARS, North Central Regional Plant Introduction Station, Ames, IA, USA 50014

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 analysis at the molecular level into complex trait dissection would enhance our understanding of mechanisms underlying phenotypic variation observed under diverse environments.

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

P195

Phenotypic characterization of sorghum nitrogen responsive gene edits using high-throughput and conventional phenotyping throughput and conventional phenotyping

(submitted by Hongyu Jin <hjin5@huskers.unl.edu>)
Full Author List: Jin, Hongyu[1 2]; Schnable, James C.[1 2]; Nolan, Alexa[4 5]; Ge, Yufeng[1 3]; Zweiner, Mackenzie[1 2]; Clemente, Thomas E.[2]; Mural, Ravi V.[1 2]; Swaminathan, Kankshita[5]; Yang, Jinliang[1 2]
[1] Center for Plant Science Innovation, University of Nebraska-Lincoln, Lincoln, NE USA 68588
[2] Department of Agronomy and Horticulture, University of Nebraska-Lincoln, Lincoln, NE USA 68583
[3] Department of Biological Engineering, University of Nebraska-Lincoln, Lincoln, NE USA 68583
[4] Department of Biological Sciences, University of Alabama in Huntsville, Huntsville, AL USA 35899
[5] Hudsonalpha Institute for Biotechnology, Huntsville, AL, USA 35806

Crop improvement over the last few decades, especially after the Green Revolution, is partially driven by the intensive application of less expensive inorganic nitrogen (N) fertilizer. However, the unsustainable use of inorganic N fertilizer in crop production decreases farming profitability and creates a series of ecological burdens. One of the long-standing goals of crop breeding is to increase crops' nitrogen use efficiency (NUE). Studies have shown a number of phenotypic variations of sorghums grown in different N conditions, including root architecture, leaf parameters, growth parameters, yield, and biochemistry traits. Additionally, previous studies showed that the demand for N varies during the sorghum developmental stages, indicating a dynamic genetic control. In our study, taking advantage of the CRISPR-based gene editing and UNL's automatic high throughput phenotyping platform, we generated five edited sorghum lines under TX430 background and phenotyped them in two N conditions from 30 days after planting to full maturity. From the imagery data, we extracted time-series plant growth traits from these edited lines as well as the wild type (i.e., TX430), such as plant height, plant width, and pixel counts, along with vegetation indices. In addition, dry weights were harvested and measured at maturity. Statistical analyses suggested the distinct N responses between some of the edited lines and the wild type were present. These N-responsive edited lines will be tested in replicated field trials and potentially be incorporated into the breeding protocol for N-resilient sorghum development.

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

P196 @Kyle__Linders

Plasticity of sorghum biomass and inflorescence traits in response to nitrogen application

(submitted by Kyle Linders <klinders@huskers.unl.edu>)
Full Author List: Linders, Kyle[2]; Mural, Ravi[2 3]; Grzybowski, Marcin W.[2 3]; Schnable, James C.[2 3]; Sigmon, Brandi[1]
[1] Department of Plant Pathology, University of Nebraska-Lincoln, Lincoln, NE, USA 68583
[2] Department of Agronomy and Horticulture, University of Nebraska-Lincoln, Lincoln, NE, USA 68583
[3] Center for Plant Science Innovation, University of Nebraska-Lincoln, Lincoln, NE, USA 68588

Nitrogen is an essential nutrient required for growth and development in plants. Insufficient nitrogen availability can reduce vegetative growth and grain yield. However, nitrogen is a costly input for farmers, is energy intensive to manufacture, and run off of excess nitrogen fertilizer impacts water quality. Compared to its close relative, maize, sorghum has much greater resilience to nitrogen and water deficit, and heat stress, allowing sorghum to be grown with fewer inputs and on marginal land. Variation in total biomass accumulation and grain yield between sorghum accessions, as well as between nitrogen conditions, can be largely explained by differences in vegetative growth and inflorescence architecture traits. Previous genome wide association studies (GWAS) in sorghum have identified genetic markers associated genes known to play roles in controlling growth and development. However, these studies have typically been conducted using field trials with “optimal” nitrogen application conditions. A set of 345 diverse inbred lines from the Sorghum Association Panel (SAP) were grown under both standard nitrogen application (N+) and no nitrogen application (N-) treatments and a range of biomass and inflorescence related traits were phenotyped, including plant height, lower and upper stem diameter, rachis length, lower and upper rachis diameter, and primary branch number. Stem volume, an approximation of biomass, was calculated from the directly measured traits. Stem volume was on average 9.6% higher for genotypes in nitrogen fertilized blocks ($p \leq 0.05$), than for genetically identical plants in no nitrogen application blocks. Within individual treatment conditions, between 58.1% and 90.7% of total variation for the measured and calculated traits could be explained by genetic factors. Genome wide association studies are currently being conducted to identify genetic markers associated with these traits in order to better understand the genetic factors involved in nitrogen stress response for potential use in breeding improved sorghum varieties.

Funding acknowledgement: Department of Energy (DOE)

P197

Protein and starch variation in heirloom corn varieties and their properties in food.

(submitted by Danielle Davis <dtdqkd@umsystems.edu>)
Full Author List: Davis, Danielle D.[1]; Salazar-Vidal, Miriam N.[1 3]; Hall, Garret[1]; Flint-Garcia, Sherry A.[1 2]
[1] University of Missouri; Columbia, MO, 65201
[2] USDA Agricultural Research Service; Columbia, MO, 65201
[3] University of California; Davis, CA,95616

Modern commercial corn varieties in the United States have limited variation between different corn types. Commercial lines are varieties bred for livestock feed, ethanol, and starch production, thus narrowing their genetic variation. Heirloom corn varieties are corn lines historically adapted to a specific region and maintained by farmers. Heirloom corn varieties have more genetic diversity because they retain characteristics that were eliminated in today's commercial corn varieties. Heirloom varieties have undesirable agronomic characteristics This breeding program will improve these agronomic characteristics in heirloom varieties while maintaining desired characteristics. The goal of my project is to determine which heirlooms can grow in Missouri, and which grain characteristics are conserved and contribute to food quality traits. Using 180 original heirloom corn varieties adapted to the United States and Mexico, 360 crosses between these varieties were made and grown in replicated experiments in Missouri during 2019 through 2021. A sample of 50 healthy and representative kernels from each experimental unit (variety*replicate*year) will be scanned with Near Infrared Spectroscopy (NIR). NIR is used as a nondestructive way to gather kernel composition data (starch and protein) allowing the kernels to be used for nixtamalization. Nixtamalization is the process of cooking grain in an alkaline solution to make amino acids available before preparing tortillas. Using a sensory panel, the culinary characteristics of the corn will be measured. By comparing the nixtamalization to the NIR results, an association between starch and protein and culinary use can be made.

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

P198 @burnsmj7

Quantification of pericarp retention of nixtamalized corn using image analysis

(submitted by Michael Burns <burns756@umn.edu>)
Full Author List: Burns, Michael J.[1]; Anderson, Nickolas[2]; Hirsch, Candice N.[1]
[1] Department of Agronomy and Plant Genetics, University of Minnesota, St. Paul, MN 55108
[2] PepsiCo R&D, St. Paul, MN 55108

Pericarp content of masa can have large effects on the machinability of the dough, the nutrition profile of the final product, and the end-user experience of appearance and flavor. To understand the biology underlying pericarp retention of nixtamalized maize, a high throughput, quantitative, and objective phenotyping method is being developed. This protocol will allow researchers to quantify pericarp retention after cooking by imaging stained kernels and utilizing computer vision approaches to determine stained and unstained pixels in the images. This method can be used to analyze maternal, environmental, and compositional effects on pericarp retention to determine which factors have the greatest effect on pericarp retention. Through the use of this tool breeders will be better able to breed for pericarp retention in food-grade cultivars, manufacturers will be able to make pass-fail decisions on samples without cooking large batches, and cooking protocols can be preemptively adjusted for differential pericarp retention rates of the raw source material that is being cooked.

Funding acknowledgement: PepsiCo R&D, Minnesota Discorvery Research and Innovation Economy (MnDRIVE), University of Minnesota Informatics Institute

P199

Quantitative genetics in the pangenome era

(submitted by Dongdong Li <ddli@iastate.edu>)
Full Author List: Li, Dongdong[1]; Escamilla, Diana M.[1]; Guo, Tingting[2]; Li, Xianran[3]; Yu, Jianming[1]
[1] Department of Agronomy, Iowa State University, Ames, IA 50011, USA
[2] National Key Laboratory of Crop Genetic Improvement, Huazhong Agricultural University, Wuhan 430070, China
[3] USDA, Agricultural Research Service, Wheat Health, Genetics, and Quality Research Unit, Pullman, WA 99164, USA

The era of pangenome, high throughput phenotyping, big data, and genome editing presents challenges and opportunities to quantitative genetics. Quantitative genetics is concerned with the inheritance of those differences among individuals that are of degree rather than of kind. A better understanding of genetic architecture of quantitative traits has fundamental significance in evolution and agriculture. Focuses of quantitative genetics have expanded from variance component analysis to design plant breeding methods to QTL mapping, marker-assisted selection, genome-wide association study, genomic selection, eQTL mapping, and transcriptome-wide association studies. In this study, we aim to review how to advance quantitative genetics research in relation to four key areas: pangenome, omnigenic model, phenotypic plasticity, and machine learning. Pangenome describes the entire set of genes within a species, both the shared core genome and the dispensable genome, providing a set of structural variations in addition to genome-wide SNPs. How to leverage pangenome to define haplotype groups to pinpoint causal polymorphisms underlying quantitative traits needs additional research. Omnigenic model stipulates that phenotypic variation of quantitative traits and complex human diseases is controlled by the direct effects of a small number of core genes and the indirect effects of a large number of peripheral genes through their regulatory effects on the expression of core genes. Omnigenic model synthesizes the findings from genome-wide association studies, gene expression analysis, and molecular pathway information, and presents a clear framework for further research. Phenotypic plasticity describes the observed phenotypic variation in living organisms under different environments. Integrated analysis of genome, environment, development, and phenotype can reveal the pattern and mechanistic interplay underlying the observed phenotype dynamics. Machine learning has been applied to integrate genomic, phenomic, and enviromic data for performance prediction. Advances in these relevant areas help move quantitative genetics forward to enrich our understanding of the genotype-environment-development-phenotype relationship.

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

P200

Relative dependency of grain yield and quality changes of maize hybrids released from 1980 to 2020 on nitrogen availability

(submitted by Lia Olmedo Pico <lolmedop@purdue.edu>)
Full Author List: Olmedo Pico, Lia B.[1]; Ruiz, Alejo[2]; King, Kyle[2]; Trifunovic, Slobodan[3]; Eudy, Douglas[3]; Archontoulis, Sotirios V.[2]; Vyn, Tony J.[1]
[1] Department of Agronomy, Purdue University; West Lafayette, IN, USA
[2] Department of Agronomy, Iowa State University; Ames, IA, USA
[3] Bayer Crop Science; St. Louis, MO, USA

Rates of maize grain yield (GY) increase due to genetic improvement have been found to be steeper under optimal conditions compared to stress environments. Additionally, increases in GY have usually been accompanied by decreases in protein concentration that are attributed to dilution processes. However, most prior studies have involved a small number of hybrids representing each decade. The objective of this research was to study how plant breeding efforts over the last four decades have led to changes in both yield and quality traits under differences in soil N availability. Four field experiments were conducted in Indiana and Illinois locations during the 2020 and 2021 growing seasons. Each individual study tested 39 Dekalb maize hybrids that were commercially released from 1983 to 2020 under two N availability levels (Low N=45-99 kg N ha^{-1} vs High N=202-246 kg N ha^{-1}). GY, kernel number (KN), kernel weight (KW), and grain concentrations of protein, oil and starch were determined at harvest. For each trait, linear models were fit in R considering Environment (combination of N level and site), Hybrid and their interaction as random effects. Hybrids BLUPs (best linear unbiased predictors) were then regressed against year of release to determine actual rates of genetic gain. GY, KN and KW increased over the last 40 years following genetic gains of 84 kg ha^{-1} $year^{-1}$, 8 grains m^{-2} $year^{-1}$, and 0.99 mg grain-1 $year^{-1}$, respectively. Conversely, quality traits showed different trends. Protein concentrations decreased significantly over time by a rate of 0.012% $year^{-1}$, while kernel oil concentrations showed no change over the last 40 years. Interestingly, starch concentration increased at a genetic gain of 0.01% $year^{-1}$, a trend that may partially explain the increase in KW. Finally, out of the 6 traits, only GY demonstrated a steeper slope of genetic gain under high N levels.

Funding acknowledgement: FFAR

P201

Screening a highly efficacious mutagenized maize library for mutants that are more susceptible or resistant to *P.maydis*

(submitted by Sendi Mejia <smejiaji@purdue.edu>)
Full Author List: Mejia, Sendi Y[1]
[1] Purdue University, West Lafayette, Indiana, 47907

Tar Spot disease of maize, caused by the fungal obligate biotroph, *Phyllachora maydis* has been rapidly progressing throughout the mid-western United States, since it's discovery in 2015. *P. maydis* is an ascomycete, and its presences in infected plants can be identified by the development of slightly raised, glossy black spots on the foliage, called stromata. *P. maydis* attacks the foliage of maize and can reduce the quality of the of silage, stover, and husks, impacting the production of grain. Due to the sudden appearance of the disease on U.S. soil, information on the biology and epidemiology of the disease is limited and reliable control methods are needed. One of the most effective strategies for disease control is the implementation of resistant cultivars. Identifying the genetic mechanisms that aid in infection is critical to uncovering sources of allelic resistance against *P. maydis.* The main objective of our research will be to investigate the genetic and physiological components that dictate maize's interaction with *P. maydis*, to develop a sustainable solution to this disease. We plan to identify susceptible and resistant related gene candidates using a diverse mutagenic population of maize. We will screen a highly efficacious EMS-mutagenized library for mutants that are more susceptible or resistant to *P. maydis* compared to the progenitor inbred B73. We will also screen maize mutants altered in host physiology, developmental pathways, and various defense responses. In addition, a large collection of cell death or lesion mimic mutants, including those with constitutively active immune responses, and those in which cell death has no obvious connection with defense response pathways. Our central hypothesis is that individuals within a mutagenic maize germplasm population will demonstrate either enhanced susceptibility or resistance to *P. maydis*, providing insight into the genetic components that assist in infection.

Funding acknowledgement: National Science Foundation (NSF)

P202

Selection for early flowering in five tropical maize populations with varying initial genetic diversity

(submitted by Eric Butoto <ebutoto@ncsu.edu>)
Full Author List: Butoto, Eric[1]; De la Cruz Larios, Lino[2]; Vanous, Adam[3]; Holland, James[1 4]
[1] Department of Crop and Soil Sciences, North Carolina State University, Raleigh, NC
[2] Department of Agricultural Production, University Center for Biological and Agricultural Sciences, University of Guadalajara, Zapopan, Jalisco, Mexico
[3] USDA-ARS, North Central Regional Plant Introduction Station, Ames, IA, USA
[4] USDA-ARS Plant Science Research Unit, Raleigh, NC, USA

Increasing genetic diversity in U.S. commercial maize will allow breeders to continue making genetic gains and provide new varieties resilient to our ever-changing environments. Tropical maize is a potential resource that can be tapped to increase genetic diversity in U.S. maize. However, adapting tropical germplasm to the temperate region requires overcoming photoperiod sensitivity, which results in the germplasm flowering very late and yielding poorly. Genetic variation within a founding population is crucial for selection to be effective in adaptation to a new environment. Measuring responses to selection in different populations with different levels of variation may provide insight into the interplay of genetic variance and selection response. We compared five different tropical populations with varying levels of initial genetic variation that were selected for earlier flowering in temperate environments for response to selection for early flowering time. Initial (unadapted) and selected (better adapted) generations of five populations (Ki14xCML254, TropicS, IA Suwan-1, IA Tuxpeño, and IA Tusón) were evaluated in three environments (Iowa, North Carolina, and Mexico) for flowering time, plant and ear height, and tassel length and branch numbers. We will test if (1) if the response to selection for early flowering time is greater in populations with larger initial standing genetic variation, (2) genotype-by-environment interactions affect responses to selection for early flowering time in a consistent manner among populations, and (3) genomic regions that exhibit allele frequency changes due to selection are consistent among populations.

Funding acknowledgement: United States Department of Agriculture (USDA)

P203

This abstract has been removed from the program.

P204 @genomeofforrest

Single-gene resolution of locally adaptive genetic variation in Mexican maize

(submitted by Forrest Li <frrli@ucdavis.edu>)
Full Author List: Li, Forrest[1]; Gates, Daniel J[2]; Buckler, Edward S[3]; Hearne, Sarah J[4]; Runcie, Daniel E[2]; Ross-Ibarra, Jeffrey[5]
[1] Department of Evolution and Ecology and Department of Plant Sciences, University of California Davis, Davis, CA, 95616, USA
[2] Department of Plant Sciences, University of California Davis, Davis, CA, 95616, USA
[3] USDA-ARS, Robert W. Holley Center for Agriculture and Health, Ithaca, NY and Institute for Genomic Diversity, Cornell University, Ithaca, NY, 14853, USA
[4] International Maize and Wheat Improvement Center (CIMMYT), El Batan, Texcoco, Estado de Mexico, 56237, Mexico
[5] Department of Evolution and Ecology, Center for Population Biology, and Genome Center, University of California Davis, Davis, CA, 95616, USA

Threats to crop production due to climate change are one of the greatest challenges facing society today. Considerable adaptive variation exists in traditional landraces, natural populations of crop wild relatives, and ex situ germplasm collections, but effective utilization of this diverse germplasm requires separating adaptive alleles from linked deleterious variants. Using a large panel of nearly 4000 collected traditional maize landraces representing a breadth of maize diversity in Central and South America, we present high-resolution genome-wide evaluation of associations between genotype and agronomically relevant phenotypic traits in 13 common gardens across different environments and elevations. We find locally adaptive alleles using observations of genotype-by-environment interactions across our common garden trails. Further, we use the package MegaLMM to perform environmental GWAS on 19 climate variables pulled from our panel of landraces to identify associations between genetic variation and the environment. In our environmental GWAS, we find that 88% of our significant peaks contain one gene, allowing us to narrow down putative targets to the single-gene resolution. Specifically, we identify a number of loci linked to climate, including the inversion *Inv4m*, previously described to be locally adaptive to highland conditions, as well as a putative heat shock protein that may provide adaptive benefit in high temperatures. Further, using MegaLMM, we use existing genotype data to predict breeding values for climate data in novel landraces and find substantial heritability for temperature-related variables. We show that there is a preponderance of functional genetic variation available in traditional landraces that can be identified through environmental and phenotypic GWAS.

Funding acknowledgement: CIMMYT

P205

SpykProps: An imaging pipeline to quantify the spike architecture in perennial ryegrass

(submitted by Joan Barreto Ortiz <jbarreto@umn.edu>)
Full Author List: Barreto Ortiz, Joan M.[1 2]; Hirsch, Candice N.[1]; Ehlke, Nancy[1]; Watkins, Eric[2]
[1] Department of Agronomy and Plant Genetics, University of Minnesota
[2] Department of Horticultural Science, University of Minnesota

Spike properties are fundamental indicators of fitness and seed productivity in grasses, and are constant selection targets throughout domestication and crop improvement. Identifying and measuring these traits, such as spikelet number, size, and distribution across the rachis, represent a challenge to breeders given the vast amount of resources required to properly phenotype and select germplasm under time constraints. Computer vision has contributed to overcoming these challenges across major grain crops, but improvement of many species is still limited by the low-throughput and subjectivity of conventional phenotyping. To address this issue, we used Python to develop SpykProps, an imaging system to quantify spike properties affecting seed yield in perennial grass (Lolium Perenne L.), a highly heterogeneous species recently domesticated for seed production. The goal of this tool is to rapidly identify spikes and spikelets in images, and quantify their color and shape properties, to facilitate selection on architectural traits affecting seed yield. Our validation indicated high accuracy (RMSE = 0.89 ± 0.49) at detecting spikes, measuring their length (R2 = 0.96), as well as high correlation between manual and automated spikelet count (rPearson = 0.78). SpykProps is able to extract over a hundred color features as well as Elliptical Fourier Descriptors from which researchers can derive latent phenotypes to more accurately determine phenology and biological seed potential while accounting for high sample variability. This tool can be leveraged to improve genetic gains for seed yield not only in perennial ryegrass but potentially other species with similar spike architecture.

P206
Standardization of phenotyping and analytical approaches to assess stalk lodging resistance in maize

(submitted by Bharath Kunduru <bkundur@clemson.edu>)
Full Author List: Kunduru, Bharath[1]; Kumar, Rohit[1]; Brar, Manwinder[1]; Stubbs, Christopher[2]; Tabaracci, Kaitlin[3]; Bokros, Norbert[4]; Cook, Douglas[5]; DeBolt, Seth[4]; Robertson, Daniel[5]; McMahan, Christopher[6]; Sekhon, Rajandeep[1]
[1] Department of Genetics and Biochemistry, Clemson University, Clemson, SC, USA 29634
[2] School of Computer Sciences and Engineering, Fairleigh Dickinson University, Teaneck, NJ, USA 07666
[3] Department of Mechanical Engineering, University of Idaho, Moscow, ID, USA 83844
[4] Department of Horticulture, University of Kentucky, Lexington, KY, USA 40546
[5] Department of Mechanical Engineering, Brigham Young University, UT, USA 84602
[6] School of Mathematics and Statistical sciences, Clemson University, Clemson, SC, USA 29634

Stalk lodging causes yield losses ranging from 2-43% in Maize (*Zea mays* L.) in different parts of the world incurring multi-billion dollar losses to the farming industry. The phenotypic expression of stalk lodging is complex and highly variable under different environments and, therefore, poses considerable challenges for reliable assessment of stalk lodging resistance (SLR). To identify plant-, organ-, and tissue-level biological and biomechanical properties of maize stalks that determine SLR, we performed a detailed characterization of 16 maize hybrids in two environments. Lack of streamlined phenotyping approaches remain a major hurdle in study of SLR, therefore, we standardized phenotyping protocols for characterizing morphological, geometric, and material properties of field-grown maize plants. Evaluation of stalk flexural stiffness, a reliable indicator of SLR, showed substantial variation for SLR among the hybrids. To understand the relative contribution of geometric and material properties, we employed modeling based on structural engineering beam theory. The parallel moment of inertia, a representation of cross-sectional geometry of stalks, varied significantly among hybrids indicating that genetic differences in major diameter, minor diameter, and rind thickness account for a portion of SLR. Contrary to tenets of beam theory, Young's modulus, an estimate of the stalk material properties, varied significantly among hybrids indicating that stalk cellular composition is an important determinant of SLR. Linear density of stalks was highly and positively correlated with SLR further strengthening a combinatorial role of geometric and material properties. Finally, to tease apart the relative contribution of individual internodes to SLR, we employed a machine learning approach that highlighted the importance of geometric and material properties of bottom internodes. Detailed analysis of stalk properties using a combination of biological and engineering approaches and the identification of genetically tractable intermediate traits will boost efforts towards genetic improvement of SLR in maize.

Funding acknowledgement: National Science Foundation (NSF)

P207 @YuRuChen15
Superior haploid inducers are attained from doubled inducer haploids

(submitted by Yu-RU Chen <yuruchen@iastate.edu>)
Full Author List: Chen, Yu-Ru[1]; Frei, Ursula K[1]; Lübberstedt, Thomas[1]
[1] Department of Agronomy, Iowa State University, Ames, IA 50011, USA

The haploid inducers in maize are used to obtain a copy of the maternal genome in the embryo by in vivo induction. The dominant markers of haploid inducers are essential factors for sorting regular F_1 and maternal haploid individuals. *R1-nj* is the typical marker of haploid inducers for haploid sorting. The transcription factor, *C1-I,* dominant inhibits the *R1-nj* functions in the biosynthesis of anthocyanin in the kernels, so inducers with *C1-I* as males could be used to easily classify the inducer haploids after crossing with the regular inducers (*mtl/zmpla1/nld, zmdmp, a1*, and *R1-nj*). The doubled haploid (DH) of inducers could be obtained then by artificial chromosome doubling. The haploid induction rate (HIR) and agronomic performance of DH lines derived from inducer F1s were evaluated in summer 2021 and 2022. The transgressive segregants in HIR, flowering time, plant height, and tassel size occurred in the DH populations; in addition, the superior inducer line had 20.1% HIR was selected, which was 1.58 times higher than its mid-parent value (12.1%). The outcomes in this study suggest that inducer doubled haploids obtained by the induction via inducers (*mtl/zmpla1/nld, zmdmp,* and *C1-I)* preserve not only the favorite minor alleles of HIR for improvement but also accelerate the inducer line development.

Funding acknowledgement: United States Department of Agriculture (USDA), Plant Sciences Institute, R.F. Baker Center for Plant Breeding, K.J. Frey Chair in Agronomy, and the Doubled Haploid Facility at Iowa State University

P208 @DorothyDSweet

Temporally resolved growth patterns reveal variability in environmental responsiveness in diverse maize panel

(submitted by Dorothy Sweet <kirsc168@umn.edu>)
Full Author List: Sweet, Dorothy D[1 2]; Tirado, Sara B[1 3]; Cooper, Julian[2]; Springer, Nathan M[3]; Hirsch, Cory D[2]; Hirsch, Candice N[1]
[1] Department of Agronomy and Plant Genetics, University of Minnesota, Saint Paul, MN 55108
[2] Department of Plant Pathology, University of Minnesota, Saint Paul, MN 55108
[3] Department of Plant and Microbial Biology, University of Minnesota, Saint Paul, MN 55108

Plant height is used in many breeding programs for assessing plant health across environments and predicting yield, which can be used in identifying superior hybrids or evaluating abiotic stress factors. This has often been measured at a single time point when plants have reached their terminal height for the season. Collection of plant height using unoccupied aerial vehicles (UAVs) is faster, allowing for measurements throughout the growing season, which could facilitate a better understanding of plant-environment interaction and responses. To assess variation in plant height and growth rate throughout development, plant height data was collected weekly for a panel of ~500 diverse inbred lines over four growing seasons. The variation in plant height throughout the season was found to be significantly explained by genotype, year, and genotype-by-year interactions throughout vegetative growth. However, the relative contributions of these different sources of variation fluctuated throughout development. Genome-wide association studies revealed many significant SNPs associated with plant height and growth rate at different parts of the growing season that were specific to certain phases of vegetative growth and would not be identified by terminal height alone. When comparing growth rates estimated from plant height to growth rates estimated from another morphological characteristic, canopy cover, we found greater stability in growth curves estimated by plant height. This potentially makes canopy cover more useful for understanding environmental modulation of overall plant growth and plant height better for understanding genotypic modulation of overall plant growth. This study demonstrates the substantial information that can be gained from high temporal resolution data in understanding how plants differentially interact with their environment and the genetic basis of that variation that is not captured from terminal plant height alone.

Funding acknowledgement: Minnesota Corn Growers Association, Bayer Crop Science

P209 @MerKhaiBurch

The perils and promise of single-gene solutions to crop yield: extraordinary claims require extraordinary evidence

(submitted by Merritt Khaipho-Burch <mbb262@cornell.edu>)
Full Author List: Khaipho-Burch, Merritt[1]; Cooper, Mark[2 3]; Crossa, Jose[4]; de Leon, Natalia[5]; Holland, James[6]; Lewis, Ramsey[6]; McCouch, Susan[1]; Murray, Seth[7]; Rabbi, Ismail[8]; Ronald, Pamela[9]; Ross-Ibarra, Jeffrey[10]; Weigel, Detlef[11]; Yan, Jianbing[12]; Buckler, Edward S.[1 13]
[1] School of Integrative Plant Sciences, Plant Breeding and Genetics Section; Cornell University; Ithaca, New York, USA 14853
[2] Queensland Alliance for Agriculture and Food Innovation (QAAFI); The University of Queensland; Brisbane, Qld 4072, Australia
[3] ARC Centre of Excellence for Plant Success in Nature and Agriculture; The University of Queensland; Brisbane, Qld 4072, Australia
[4] International Maize and Wheat Improvement Center (CIMMYT); Carretera México-Veracruz Km. 45, El Batán, Texcoco 56237, Edo. de Mexico, Mexico
[5] Department of Agronomy; University of Wisconsin; Madison, WI, USA 53706
[6] Department of Crop and Soil Sciences; North Carolina State University; Raleigh, NC, USA 27539
[7] Department of Soil and Crop Sciences; Texas A&M University; College Station, Texas, USA 77843
[8] International Institute of Tropical Agriculture (IITA); Ibadan, Nigeria
[9] Department of Plant Pathology and the Genome Center; UC Davis; Davis, CA, USA 95616
[10] Department of Evolution & Ecology, Center for Population Biology and the Genome Center; University of California; Davis, CA, USA 95616
[11] Department of Molecular Biology; Max Planck Institute for Biology; Tübingen, 72076 Tübingen, Germany
[12] National Key Laboratory of Crop Genetic Improvement; Huazhong Agricultural University; Wuhan, 430070, China
[13] USDA-ARS; Ithaca, NY, USA 14853

Agriculture relies on plant breeding and genetics to deliver the best crop varieties and agronomic practices that will deploy results across nearly a billion hectares of cropland. We regularly see industry delivering on this objective, although optimized for their goals and limited geographies. However, many public sector efforts responsible for feeding most of the planet are not meeting these objectives or working effectively across disciplines. A key example is repeated publications on single genes, multiple gene constructs, or similar edits that claim to confer incredible yield increases. Many of these publications are flawed in how they measure and report yield. Often, they lack replication across environments, have low sample sizes, compare yield estimates in non-commercially competitive germplasm, and report on experiment-specific yields that massively underperform as compared to real-world local or global yields. Here, we detail common issues that arise and describe how such findings, when published in high-profile journals, skew global agricultural funding away from proven plant breeding methods.
To address these issues, we suggest approaches for researchers and reviewers to use when evaluating the impact of single genes on crop yield, including:Robustly measure crop-relevant yield, not plant-level yield.Create field designs that pay attention to inter-plant competition and genotype-by-environment interactions.Use elite germplasm.Prioritize genes that evolution may have missed or whose variation has been exhausted within elite germplasm.Develop collaborations and use public sector frameworks such as the Genome to Fields Initiative to test changes at scale.Accurate measurement and reporting of crop productivity have drastic consequences for feeding the planet. Due to the impact that many of these problematic yield studies have on setting global food policy and subsequent economic investments, there is no more important time to collaborate as a community to accurately measure, improve, and deliver on crop resilience and yield.

Funding acknowledgement: United States Department of Agriculture (USDA)

P210

Trans-eQTL contributes to gene expression heterosis in maize

(submitted by Gen Xu <gxu6@unl.edu>)
Full Author List: Xu, Gen[1]; Yang, Xuerong[2]; Liu, Hongjun[2]; Yang, Jinliang[1]
[1] Department of Agronomy and Horticulture, University of Nebraska-Lincoln, Lincoln, NE 68583, USA
[2] State Key Laboratory of Crop Biology, College of Life Sciences, Shandong Agricultural University, Tai'an, China

Heterosis, or hybrid vigor, is one of the most critical genetic phenomena and has been successfully applied in agricultural practices to achieve high yielding for crop production. Although the molecular basis for heterosis has been extensively studied, transcriptomic contributions to heterosis remain largely elusive. Here we report an integrative analysis of seedling traits, adult agronomic traits, and molecular traits (transcriptomic data) of 200 maize inbreds and their 600 F1 hybrids. We found the majority of hybrids (85.3%) have more active genes than their inbred parents, whereas 10,622 (49%) genes display positive mid-parent heterosis (MPH) for gene expression, and 5,495 (25.3%) genes show negative MPH. Results showed that the number of single-parent expression (SPE) genes is significantly associated with MPH for most seedling traits. Heritability analysis suggested that the heterosis traits (i.e., MPH) are more heritable than traits *per se*, especially for the gene expression traits. After partitioning the genetic variance into additive and dominance variances, we found that dominance variances were positively correlated with positive MPH of gene expression. By examining the count of deleterious mutations in the hybrids, we further revealed that the accumulation of deleterious mutations at the gene body slightly contributes to the MPH of gene expression. Results from transcriptome-wide association studies (TWAS) demonstrated that the heterosis of molecular traits is mainly regulated by *trans*-eQTL. Furthermore, we identified 911 eQTL hotspots that regulate the MPH of gene expression, and these eQTL hotspots tend to locate at genomic regions with higher recombination rates and genetic diversity. Taken together, our results suggest *trans*-eQTL contributes to gene expression heterosis and provides insights into the genetic basis of heterosis for the agronomic traits at the gene expression level.

Funding acknowledgement: United States Department of Agriculture (USDA)

P211

Undertaker: a stereo imaging system to explore latent canopy traits

(submitted by Zhongjie Ji <jizhongj@msu.edu>)
Full Author List: Ji, Zhongjie[1]; Thompson, Addie[1]
[1] Department of Plant, Soil & Microbial Sciences; Michigan State University; East Lansing, MI 48824

Maize canopy architecture impacts yield through differences in light interception as well as weed suppression. Robust quantification of canopy structure is very complicated, as the overall structure is the result of several leaf traits, such as leaf number, size, shape, curvature, and inclination. The measurement of leaves within the canopy is not easy, and there are many interactions between them. Recent image and sensor based high throughput phenotyping enables the exploration of more complex canopy traits that may help to summarize overall canopy structure. Aerial-based approaches, though more straightforward, are only able to obtain information on the canopy surface. Our lab developed a device called the undertaker, consisting of two GoPro cameras, to capture image-based latent canopy features. We used the undertaker to collect three years of data in the Michigan Genomes to Fields project from 2019 to 2021. All images were taken 10 days after the peak of anthesis to avoid pollen and anthers falling on the cameras. To process the images, the simultaneous photos from left and right cameras were first calibrated and undistorted. Then the calibrated images were used to generate disparity maps. In this study, we attempted to use the disparity maps from single channel, RGB, and synthetic RGB-D (depth) data and machine learning to predict hand-measured leaf and canopy traits. Novel traits can then be extracted from the images that describe canopy features.

Funding acknowledgement: Corn Marketing and Promotion of Michigan, MSU Plant Resilience Institute

P212

Using the human gut microbiome as a phenotype of sorghum in a genetic mapping study

(submitted by Nate Korth <nkorth@huskers.unl.edu>)
Full Author List: Korth, Nate[1 2 3 4]; Yang, Qinnan[1 2]; Van Haute, Mallory[1 2]; Tross, Michael[3 4]; Mural, Ravi[3 4]; Schnable, James C.[2 3 4]; Benson, Andrew K.[1 2]
[1] Department of Food Science and Technology; University of Nebraska-Lincoln; Lincoln, NE 68508, USA
[2] Nebraska Food for Health Center; University of Nebraska-Lincoln; Lincoln, NE 68508, USA
[3] Department of Agronomy and Horticulture; University of Nebraska-Lincoln; Lincoln, NE 68508, USA
[4] Center for Plant Science Innovation; University of Nebraska-Lincoln; Lincoln, NE 68508, USA

The growing epidemic of obesity and related diseases shares casual features of western dietary patterns and dysbiosis of the human gut microbiome. Tens of trillions of microorganisms comprise the gut microbiome which contributes to bodily functions such as digestion and development of the immune system. Species composition of the gut microbiome is heavily influenced by diet. Staple grains that provide a significant percentage of calories consumed by humans are comprised of few elite hybrid lines selected primarily for yield and resilience. While these traits are vital, an opportunity exists to study and improve nutritional qualities of grains, including grain components that are substrates for desirable gut microbes. We employed a quantitative genetics approach to identify novel sorghum traits within existing genetic diversity that affect the composition of human gut microbe communities. We developed an automated *in vitro* microbiome screening platform as a high-throughput method for phenotyping the effects of grains on gut microbes. We demonstrated sorghum population structure can explain variation in bacterial response in the microbiomes of eight humans. In a genome-wide analysis, we identified eleven multiple-effect loci (MEL) in the sorghum genome where variation affects multiple taxa of gut microbes from two distinct humans. Four of these MEL overlap with MEL for gut microbiome detected in a previous study of a RIL population and expand the catalogue of MEL in sorghum that can influence seed composition which can have major effects on the gut microbiome. Genome-wide mapping of biochemical and agronomic traits in the SAP population revealed candidate seed traits likely causal for the microbiome associations. This work demonstrates that genetic factors affecting sorghum seed can drive significant effects on gut microbes, particularly on those considered to be beneficial. Understanding these relationships will enable targeted breeding strategies that can improve human health through modulation of the gut microbiome.

Funding acknowledgement: FFAR, Nebraska Food for Health Center, Raikes Foundation

P213

Using variance component analysis to connect single parent expression to phenotype

(submitted by Sophia Schmidt <sophie7@iastate.edu>)
Full Author List: Schmidt, Sophia G[1]; Snodgrass, Samantha J[1]; Hufford, Matthew B[1]
[1] EEOB Iowa State University; Ames, Iowa, United States 50010

The genetic mechanism underlying hybrid vigor, the phenomenon of hybrids far outperforming their parents, remains an active area of research. Single Parent Expression (SPE) is the most extreme differential expression and has been linked to hybrid vigor. A gene displays SPE if one parent and the hybrid both show expression while the other parent does not. Thus, the hybrid plants have more genes being expressed than either parent, which may contribute to the hybrid's superior performance. However, there has been limited research connecting SPE patterns directly to phenotypes. Here we attempt to bridge this gap using published datasets of the NAM RIL population for both genotypes and 143 phenotypes as well as published sets of genes that have shown SPE patterns across various crosses and tissue types to conduct a variance component analysis (VCAP). A VCAP estimates the amount of additive genetic variation a subset of the genome contributes to the overall phenotypic variation observed. We will partition the genome into genes that always display SPE, vary in SPE across different crosses or tissue types, and those that never display SPE. This will allow us to clarify relationships between SPE patterns and overall trait heritability as well as particular types of traits (ex. plant architecture, flowering time, metabolites, etc.). Through this analysis we hope to better understand the overall connection between genes that display SPE patterns and traits of interest in maize. This will allow us to develop more targeted research questions about SPE patterns and how they may be used to promote hybrid vigor.

Funding acknowledgement: National Science Foundation (NSF)

P214

Validation of a root system architecture QTL and insight into the impact of structural variants on GWAS in maize

(submitted by Patrick Woods <patrick.woods@colostate.edu>)
Full Author List: Woods, Patrick[1]; Mullen, Jack[1]; McKay, John[1]
[1] Colorado State University; Fort Collins, CO, 80523

A long-term goal of agricultural genetics is to understand the relative roles that different types of genomic variation such as single nucleotide polymorphisms (SNPs) and structural variants (SV) play in shaping the genetic basis of complex traits in crops. Despite this goal, current approaches for identifying causal genetic variation such as genome wide association studies (GWAS) and interval mapping (IM) often neglect to incorporate SVs into their analyses. Neglecting the incorporation of SVs into analyses warrants concern from both applied and theoretical genetic perspectives on gene mapping for complex traits. We are studying the impact of exclusion/inclusion of SVs in both GWAS and IM for identifying candidate genes controlling drought adaptive root traits in maize. In this study, we measured the root pulling force (RPF) in the B73 x CML69 maize NAM RIL population to validate the biological significance of an root trait quantitative trait locus (QTL) identified in our previous GWAS study (Indeterminant Domain 14, IDD14). RPF is the physical force required to extract an adult maize plant from the soil. To improve the applicability of RPF in large-scale field experiments, we designed and implemented a novel high throughput phenotyping tractor capable of automating RPF data acquisition. Through our analyses, we address outstanding applied and theoretical genetics questions by 1) determining that an SV potentially underlies one RSA QTL we identified previously, 2) re-assessing this QTL's significance by incorporating formal tests of the SV in QTL models, and 3) demonstrating that SVs are significantly associated with publicly available SNPs called throughout the maize genome.

Gene / Gene Models described: *IDD14*; Zm00001d048722
Funding acknowledgement: United States Department of Agriculture (USDA)

P215

ZmIRA1, a new mediator between Root System Architecture (RSA) and nitrogen metabolism

(submitted by Michelle Cho <michelle.s.cho@wustl.edu>)
Full Author List: Cho, Michelle S.[1 2]; Liu, Zhengbin[3]; Yang, Bing[2 4]; Gunn, Shayla[2]; Duncan, Keith[2]; Sellers, Mitchell[2]; Lebow, Clara[2]; Moose, Stephen P.[5]; Topp, Christopher N.[2]
[1] Division of Biology and Biomedical Studies, Department of Biology, Washington University in St. Louis, St. Louis, MO, 63110 USA
[2] Donald Danforth Plant Science Center, St. Louis, MO, 63132 USA
[3] Partek Inc, Chesterfield, MO, 63005 USA
[4] Division of Plant Sciences, Bond Life Sciences Center, University of Missouri, Columbia, MO 65211, USA.
[5] Department of Crop Sciences, University of Illinois Urbana-Champaign, Urbana, IL 61801, USA.

Maize root system architectures have been indirectly altered during breeding, but little is known of the nature of these changes or implications for root system functions. We leveraged the Illinois Long Term Protein Selection Strains (ILTPS) to understand how over 100 years of recurrent selection for (nitrogen-rich) seed protein content may have affected root structure and nitrogen metabolism of plants. Using a mapping population derived from ILTPS, the Illinois Protein Strain Recombinant Inbreds (IPSRIs), we performed a Genome-Wide Association study of root crown traits from field-grown plants. A Quantitative Trait Locus (QTL) was identified, and a single candidate gene was identified in the local region of linkage disequilibrium, which we refer to as IRA1 (Ideal Root System Architecture 1). Sequencing of related Illinois High and Low Protein inbred lines (IHP and ILP respectively) revealed promoter indels. Deletions in ILP promoter sequence reduced IRA1 expression in root tissues. We used a small indel to genotype a subset of the IPSRI population for either IHP-like or ILP-like alleles. These were grown in a field experiment to assess the effects of IHP- or ILP-like alleles on 3D root crown architecture as measured from X-ray tomography-generated models and a custom feature extraction pipeline. The presence of the ILP-like allele significantly increased the solidity of the root crowns, which is a measure of the thoroughness of root system exploring the volume of soil it inhabits. Changes in solidity are a well-known response to nitrogen sensing. In 2022 we conducted another field experiment under normal and low-nitrogen fertilization to assess potential effects on nitrogen relations. Meanwhile, we used CRISPR-Cas9 to target IRA1 and a paralog in B73. We isolated mutants with stop and frameshift mutations in both genes and experiments are underway to evaluate their impact on root system architecture and nitrogen sensing, uptake, and metabolism.

P216

metaGE: Investigating genotype x environment interactions through GWAS meta-analysis

(submitted by Alain Charcosset <alain.charcosset@inrae.fr>)
Full Author List: De Walsche, Annaïg[1 2]; Charcosset, Alain[1]; Mary-Huard, Tristan[1 2]
[1] INRAE GQE Le Moulon; Gif-sur-Yvette; France
[2] AgroParisTech MIA Paris; Palaiseau; France

Multi-Environment Trials (MET) have been widely adopted in plant genetics to dissect the genetic components underlying the Gene-by-Environment interactions. While promising, the statistical modelling for genome-wide association studies (GWAS) of MET data is significantly more complex than for single environment studies. Most current methods rely on approximate test procedures to reduce the computational burden. As an alternative, we introduce metaGE, a flexible and computationally efficient meta-analysis (MA) approach for the joint analysis of any MET GWAS or multi-parental population experiment. The metaGE approach accounts for both the heterogeneity of QTL effects across environments and the correlation between GWAS summary statistics acquired on the same (or related) set of genotypes across different environments. The applications of our MA approach identified new candidate QTLs and provided valuable insight into the genetic architecture of several complex traits compared to previous GWA analyses in 3 species and a multi-parent MET population. The whole procedure is available as an R package.

P217

A role for RdDM in susceptibility to paramutation at the maize *BOOSTER1* locus

(submitted by Kevin Peek <k.peek@uva.nl>)
Full Author List: Peek, Kevin[1]; Lauss, Kathrin[1]; Bader, Rechien[1]; Lee, Tzuu-fen[2]; Meyers, Blake[2 3]; Stam, Maike[1]
[1] Swammerdam Institute for Life Sciences; The Netherlands; Amsterdam; 1098XH
[2] Department of Plant and Soil Sciences; USA; Newark; DE 19711
[3] Donald Danforth Plant Science Center; USA; St. Louis; MO 63132

Paramutation is defined as *in trans* communication between alleles, whereby the silent epigenetic state is heritably transferred to an active (epi)allele. When the active copy is silenced it becomes able to induce silencing of other active copies (secondary paramutation). Paramutation of the *BOOSTER1* (*b1*) locus in maize (*Zea mays*) is one the best studied systems. *B1* encodes a transcription factor in the anthocyanin biosynthesis pathway. Two epialleles participate in *b1* paramutation: the lowly expressed *B'* allele and the highly expressed *B-INTENSE* (*B-I*) allele[1]. A seven-times repeated 853bp sequence located about 100kb upstream of *b1* is required for paramutation and high expression of *b1*[2]. The region required for paramutation was mapped to the 5' half of the repeat unit[3]. Based on DNA methylation levels, two differentially methylated regions (DMR1 and DMR2), were identified[4]. At the inactive *B'* epiallele, DMR1 and DMR2 are highly methylated in CG and CHG context, whereby DMR1 results in low 24nt siRNAs levels. At the active *B-I* epiallele, DMR1 is unmethylated while DMR2 shows high CG, CHG and CHH methylation and results in relatively high 24nt siRNAs levels[4]. Counterintuitively, these findings implicate the presence of the RNA-directed DNA Methylation (RdDM) machinery on DMR2 of the *B-I* epiallele. We hypothesize that DMR2 of *B-I* causes susceptibility to paramutation. An adapted RRBS method[5] provided first indications that upon paramutation of *B-I*, DNA methylation is spreading from DMR2 into DMR1. This raises an interesting question about the paramutation requirements; Is an RdDM-targeted region on an active allele required to be sensitive to paramutation?

1. Coe, E. H. *PNAS* **45**, 828 (1959).
2. Stam, M. *et al*. *Genes Dev* **16**, 1906 (2002).
3. Belele, C. L. *et al. PLoS Genet* **9**, e1003773 (2013).
4. Lauss, K. University of Amsterdam (2017).
5. Edelmann, S. & Scholten, S. *MMB* **1675**, 45 (2018).

Gene / Gene Models described: *b1 - colored plant1*; Zm00001eb074320

Funding acknowledgement: Topsector TKI

P218
Abstract removed at the request of the author

P219 @Bimala25
Harnessing the helitron
(submitted by Bimala Acharya <bacharya@iastate.edu>)
Full Author List: Acharya, Bimala[1]; Lisch, Damon[2]; Anderson, Sarah[1]
[1] Department of Genetics, Development and Cell Biology, Iowa State University, Ames, IA 50011, USA
[2] Department of Botany and Plant Pathology, Purdue University, West Lafayette, Indiana, IN 47907, USA

Transposable elements are the DNA sequences that can move from one place to another in the genome. They are found in both prokaryotes and eukaryotes. There are two classes of transposable elements: Class I Retrotransposon and Class II DNA transposon. The maize genome contains a large number of LTR retrotransposons, TIR DNA transposons, and Helitron DNA transposons. Helitrons were first identified in 2001 by computational analysis of the genome of Arabidopsis, Rice, and *Caenorhabditis elegans*. In maize, helitrons contribute to genomic diversity by capturing fragments of genes and moving these fragments to new positions in the genome. Helitrons are unusual in that they lack the structural features of TIR transposons and LTR retrotransposons, including both direct or inverted repeats at the TE ends and target site duplications flanking insertions. This makes helitrons especially tricky to annotate, which had led to inaccuracies in annotation of large numbers of putative genes. Accurate annotation of helitrons is critical to understanding the origin of gene and pseudo-gene sequences found interspersed throughout the genome. Here, we propose a gene-centric method for identifying helitron insertions where we first identify trans duplicated gene fragments genome-wide, then implement filtering and characterization steps to identify TE ends and family structure. The vast majority of gene fragments contained within helitrons are likely to have lost their original functions due to fragmentation and epigenetic silencing. However, the presence of a vast pool of transduplicated genic DNA is likely to have impacted evolution of the host gene function in surprising ways. With this work, we aim to better characterize the structure and distribution of helitrons to better understand the functional impact that these enigmatic transposable elements have had on the evolution of the maize genome as a whole.

Funding acknowledgement: Iowa State University

P220 @Clore_IV

LTR Predictor: A tool to identify LTR retrotransposon insertions in long-read genome sequencing data

(submitted by William Clore <whclore@iastate.edu>)
Full Author List: Clore, William H[1]; Guo, Yuxiang[1]; Springer, Nathan M[2]; Anderson, Sarah N[1]
[1] Department of Genetics, Development and Cell Biology, Iowa State University, Ames, IA, USA 50011
[2] Department of Plant and Microbial Biology, University of Minnesota, Saint Paul, MN, 55108, USA

Long Terminal Repeat (LTR) retrotransposons are the most abundant type of transposable element (TE) found in the maize genome, characterized by having long terminal repeats on either side of a coding sequence or deleted coding sequence. LTR retrotransposons make up the majority of the maize genome, but only a handful of new insertions have ever been identified in controlled experiments. With recent advances in long-read sequencing technologies, we are now able to fully sequence single molecules that include new insertions of long TEs along with enough surrounding DNA to map the insertion with high confidence. However, existing tools used to annotate LTR retrotransposons based on structural features could not be applied in this context. Here we describe a new tool, LTR Predictor, which can be used to identify LTR retrotransposon candidates from inserted sequences defined through long-read sequencing. LTR Predictor searches sequences for long terminal repeats and primer binding sites, filters out insertions that are too short or composed entirely of simple repeats, then creates output files in table and graphical formats for high-quality candidates. Since this tool relies on structural features rather than homology, prior knowledge of LTR retrotransposons already present in the genome is not required. This tool will aid in discovery of novel LTR retrotransposon insertions in plant genomes, paving the way for downstream studies on the consequences of TE insertions on genome function.

Funding acknowledgement: Iowa State University College of Liberal Arts and Sciences

P221

Limited contribution of transposable elements to regulatory adaptation in maize inbreds and hybrids

(submitted by Merritt Khaipho-Burch <mbb262@cornell.edu>)
Full Author List: Khaipho-Burch, Merritt[1]; Stitzer, Michelle[2]; Berthel, Ana[2]; Casstevens, Terry[2]; Miller, Zack[2]; Romay, M. Cinta[2]; Buckler, Edward[1 2 3]
[1] Section of Plant Breeding and Genetics, Cornell University, Ithaca, NY, USA 14853
[2] Institute for Genomic Diversity, Cornell University, Ithaca, NY, USA 14853
[3] USDA-ARS, Ithaca, NY, USA 14853

Transposable elements (TEs) contribute to ~85% of the maize genome and have been shown to impact genome structure and gene regulation by rearranging, adding, disrupting, or silencing endogenous promoters. In maize, large-effect TE insertions have implications on phenotypes, such as flowering time and the shift to apical dominance. The effect that TEs have on the expression of non-domestication genes during normal plant development is less understood and typically confounded by trans-regulatory sequences, which are unlinked from the focal gene and act through diffusible products. Because cis-regulatory sequences are linked to the gene they impact, we partition the variance in gene expression into the highly heritable cis-only component, allele-specific expression, and measured expression to determine which TEs cause substantial effects on gene expression. Using RNAseq data generated in inbred and hybrid genotypes created from the founders of the maize Nested Association Mapping panel, we found that less than 1% of all cis-regulatory transposable element insertions regulate or disrupt gene expression in non-stressed tissues. We then characterized the types of genes impacted through gene ontology enrichment and described other genomic features associated with these expression changes. These patterns can guide plant breeders on how to design high-yielding maize varieties more suited to new environments and conditions.

Funding acknowledgement: United States Department of Agriculture (USDA)

P222

Maizecode: DNA regulatory elements in maize and teosinte inbreds provide insight into maize domestication

(submitted by Jonathan Cahn <cahnjonathan@gmail.com>)
Full Author List: Cahn, Jonathan[1]; Regulski, Michael[2]; Lynn, Jason[2]; Ernst, Evan[1]; Ramakrishnan, Srividya[3]; Chougule, Kapeel[2]; Xiaosa, Xu[2]; Drenkow, Jorg[2]; Micklos, David[4]; Birnbaum, Kenneth D.[5]; McCombie, W. Richard[2]; Schatz, Michael C.[3]; Ware, Doreen[2]; Jackson, David[2]; Gingeras, Thomas R.[2]; Martienssen, Robert A.[1]
[1] HHMI-Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
[2] Cold Spring Harbor Laboratory, Cold SpringHarbor, NY
[3] Johns Hopkins University, Baltimore, MD
[4] DNA Learning Center, Cold Spring Harbor, NY
[5] New York University, New York, NY

Early maize lines were domesticated from *Teosinte parviglumis* (*Z.mays parviglumis*), with subsequent introgressions from neighboring *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 developed an automatized computational pipeline to integrate these datasets as well as publicly available data. This pipeline generates metrics and outputs for both quality control and functional analyses and it can also be applied to other species.
We identified regulatory regions that emerged during the domestication process and are responsible for the tissue-specific expression of developmental genes. 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, corroborating the very distinct morphological and physiological differences between maize and teosinte. This integrative analysis highlighted the role of epigenetic data in helping refine the functional annotation of the genome, notably by identifying distal “super enhancers” similar to those found in animal genomes. With this study, we hope to provide the maize community with a framework for a collaborative effort that follows the footsteps of the ENCODE project in order to better understand and potentially improve the regulatory landscape of the maize genome.

Funding acknowledgement: National Science Foundation (NSF)

P223

Mdr1 demethylase and the intersection between the epigenome, genomic imprinting, and transposable elements in maize endosperm

(submitted by Kaitlin Higgins <kaitlinj@iastate.edu>)
Full Author List: Higgins, Kaitlin M[1]; Gent, Jonathan[2]; Kim, Dong won[2]; Anderson, Sarah N[1]
[1] Department of Genetics, Development, and Cell Biology, Iowa State University, Ames, IA, 50010
[2] Department of Genetics; University of Georgia; Athens, GA, USA 30602

In 1970 Jerry Kermicle published the first evidence for a phenomenon now known as genomic imprinting using a mutant of R1. Genomic imprinting is the expression of an allele dependent on the parent of origin. Since then, theories about the evolutionary underpinnings, and the search for the molecular mechanisms causing imprinting have intrigued scientists. Here we present new findings that provide insights into the role of maternal de-repression of R1(Mdr1), a regulator of R1, in maize endosperm. In 2022, Mdr1 was mapped. The mdr1 mutant was first discovered through its role in imprinting. In WT plants, Mdr1 demethylates the maternal allele of R1, setting up maternal expression in the endosperm. While in the mutant, R1 remains methylated and loses expression in the endosperm. To test the genome-wide effects of mdr1 on expression, we performed RNA-seq on mdr1 mutant and wild-type endosperm at 14 days after pollination. This revealed 97 genes, and 89 transposable elements(TEs), that are differentially expressed in the mutant. All but one gene and three TEs are down-regulated in the mutant, as was previously seen with R1. These down-regulated genes have many shared associations with other genomic datasets, including an enrichment for overlap with differentially methylated regions (DMRs) between mutant and wild type, overlap with a helitron family with several members which are differentially expressed in the mutant, and genes that are maternally expressed (imprinted) in the endosperm. To further explore the role mdr1 plays in the endosperm epigenome we are assessing the histone landscape using CUT&Tag. Together, these data suggest MDR1-dependent demethylation contributes to the distinct transcriptomic environment in endosperm, and to epigenome changes associated with maternally expressed genes and transposable elements.

Gene / Gene Models described: *mdr1*; GRMZM2G422464
Funding acknowledgement: National Science Foundation (NSF)

P224 @NathanSCatlin

Methods for detecting TE PAVs in a maize diversity panel

(submitted by Nathan Catlin <catlinna@msu.edu>)
Full Author List: Catlin, Nathan S.[1 2]; Platts, Adrian E.[1]; Munasinghe, Manisha[3]; Springer, Nathan M.[3]; Josephs, Emily B.[1 2]
[1] Department of Plant Biology, Michigan State University
[2] Ecology, Evolution, and Behavior Program, Michigan State University
[3] Department of Plant and Microbial Biology, University of Minnesota

Over 80% of the maize genome originated from transposable elements (TEs), making maize an important but challenging system to study how TEs contribute to genome evolution. Recent advances in bioinformatic approaches using short read genomic sequencing allow scientists to quantify the variation in TE content between maize assembled genomes. However, without long-read sequencing, the ability to accurately identify maize TE polymorphisms in larger maize populations remains challenging. Since TE insertions and deletions are a type of structural variant (SV), we propose to leverage characterized SVs from whole genome alignments between the 25 maize nested association mapping (NAM) inbred genomes to predict the location of polymorphic TEs using short read alignments. First, SVs are identified for each NAM genome mapped to B73. Next, two alleles are extracted per SV: an SV-present allele including the SV sequence along with 300 bp flanking syntenic regions and an SV-absent allele with 300 bp flanking syntenic regions surrounding the SV site. Short reads from a genotype of interest are then aligned to these pseudo-reference alleles to predict if the SV is present or absent in this genotype based on contiguous or split read mapping outcomes. Preliminary results using short read data from two maize genotypes with known SV calls show split read mapping across SV junctions for reads from SV-absent genomes, demonstrating our ability to call TE polymorphisms using short read data. Next, we will apply this method to short read data from the Goodman association panel. Ultimately, we will quantify TE and SV presence/absence variation across 277 maize genotypes and associate TE polymorphism with corresponding phenotypic and RNA-Seq data.

Funding acknowledgement: National Science Foundation (NSF)

P225

Natural methylation epialleles correlate with gene expression in maize

(submitted by Jonathan Gent <gent@uga.edu>)
Full Author List: Yeng, Yibing[2]; Dawe, R Kelly[1 2]; Gent, Jonathan[1]
[1] University of Georgia, Department of Plant Biology; Athens, GA 30602
[2] University of Georgia, Department of Genetics; Athens, GA 30602

DNA methylation (5-methylcytosine) represses transposon activity and contributes to inaccessible chromatin structure of repetitive DNA in plants. It is depleted from cis regulatory elements in and near genes, but in some genes it is present in the gene body including exons. Methylation in exons solely in the CG context is called gene body methylation (gbM). Methylation in exons in both CG and non-CG contexts is called TE-like methylation (teM). To develop a broader understanding of methylation in maize genes, we utilized recent genome assemblies, gene annotations, transcription data, and methylome data to decipher common patterns of gene methylation. We found that teM genes are mainly silent across plant tissues, are limited to specific maize stocks, and exhibit evidence of annotation errors. We used these data to flag all teM genes in the 26 NAM founder genome assemblies (on average 3,693 genes, 9% of total). In contrast to teM, gbM genes are broadly expressed across tissues. We found that they exist in a continuum of CG methylation levels without a clear demarcation between unmethylated genes and gbM genes. Analysis of expression levels across diverse maize stocks revealed a weak but highly significant positive correlation between gbM and gene expression. gbM epialleles were associated with an approximately 3% increase in steady-state expression level relative to unmethylated epialleles. We hypothesize based on these data that gbM can contribute toward broad and robust gene expression.

Funding acknowledgement: National Science Foundation (NSF)

P226

Predicting the fine scale crossover landscape in maize

(submitted by Ruth Epstein <rke27@cornell.edu>)
Full Author List: Epstein, Ruth[1]; Pawlowski, Wojtek[1]
[1] Cornell University; Ithaca, NY, 14850

Crossing-over (CO) rate is heterogeneous along chromosomes in maize and exhibits a distinct “U”-shaped pattern, where the highest recombination rates are in the sub-telomeric regions, and the lowest rates are around the centromere. The specific chromatin and sequence environments that lead to this “U”-shaped distribution have not been fully elucidated. Previous studies have demonstrated that CO presence or absence in maize can be predicted with high accuracy using machine learning based on local chromatin features. However, understanding CO site location may still offer limited insight into the features that regulate CO rate heterogeneity. In order to investigate what influences CO rate heterogeneity on a fine scale, a large number of COs mapped with high resolution are needed, which would necessitate using a massive experimental population. To sidestep this problem, we used historical data to complete an identity-by-descent (IBD) analysis between the NAM *de novo* assemblies which resulted in about 100,000 IBD ends, or inferred past COs, on chromosome 1. The resulting inferred COs were then used as input into a machine learning (ML) model utilized to predict recombination rate at a 10kb resolution. The ML model performed well and is the first model developed to predict recombination rate, rather than binary presence/absence of COs. Furthermore, the top contributing features to the ML model were identified to be H2A.Z, distance to centromere, and level of CG methylation. In downstream analyses, the developed ML model can be used to predict recombination rate in unseen diverse germplasm or in mutants that alter chromatin dynamics.

Funding acknowledgement: National Science Foundation (NSF)

P227 @KochLabUF

See what's new at UniformMu

(submitted by Nadia Mourad Silva <nmourad@ufl.edu>)
Full Author List: Saunders, Jonathan W.[1]; Gray, Katherine E.[1]; Mourad Silva, Nadia N.[1]; Guan, Jiahn-Chou[1]; Suzuki, Masaharu[1]; Gustin, Jeffery L.[2]; Zimmerman, Shane A.[2]; McCarty, Donald R.[1]; Sachs, Martin M.[2]; Koch, Karen E.[1]
[1] University of Florida, Department of Horticultural Sciences, Gainesville, FL, USA 32611
[2] USDA-ARS Soybean/Maize Germplasm, Pathology & Genetics Research Unit, Urbana, IL, USA 61801

Previously unexplored sources of new mutants include lines with phenotypes arising from Mu-active populations other than UniformMu. Some of these are currently curated by the Maize Genetics Cooperation Stock Center and include mutations from Robertson's original population and others that alter B-vitamin content, endosperm structure, embryo phenotypes, and more. Here we report results of pilot studies aimed at identifying causal genes in these materials. A thorough screen of databases and historic data indicated that there are hundreds of mutant phenotypes with unidentified causal genes. Because these were generated in Mu-active populations, we surmise that a substantial fraction will be Mu-tagged and thus accessible to the Mu-seq-UniformMu pipeline for linking phenotype to genotype. We focused on those mutants conferring seed phenotypes, with emphasis on small, shrunken, embryo-lethal, and other types of defective kernels. All selections showed clear heritability and allowed establishment of a stable, Mu-off state, typically in less than three generations. We anticipate that Mu-seq analysis of these and other unexplored lines will identify mutants not yet observed in the UniformMu resource given differences we have observed between Mu-active populations. This approach will also allow delineation of allelic relationships within mutant groups, most of which include subtle to pronounced variations. In addition to enhancing the array of currently available, Mu-tagged mutants, the value of other extant resources can also be enhanced by this phenotype-to-genotype effort.

Funding acknowledgement: National Science Foundation (NSF)

P228

Silencing release at *b1* gene in RdDM mutants is associated with reduced H3K9me2 and H3K27me2 rather than DNA methylation

(submitted by Maike Stam <m.e.stam@uva.nl>)
Full Author List: Hovel, Iris[1]; Bader, Rechien[1]; Louwers, Marieke[1]; Lauss, Kathrin[1]; Haring, Max[1]; Stam, Maike[1]
[1] Swammerdam Institute for Life Sciences, University of Amsterdam, Science Park 904, 1098 XH Amsterdam, The Netherlands

Paramutation, the transfer of heritable silencing information between two alleles, results in the silencing of a susceptible allele[1,2]. Paramutation requires multiple components of the RNA-directed DNA methylation (RdDM) pathway, which is characterized by 24nt siRNAs and CHH methylation. With paramutation at the maize *b1* gene, affecting plant pigmentation, the low expressed *B'* epiallele heritably changes the high expressed *B-I* epiallele into *B'* with 100% frequency. A hepta-repeat 100kb upstream of *b1* is required for paramutation and high *b1* expression[3]. Intriguingly, mutants of different RdDM components preventing paramutation have opposing effects on the chromatin structure at the *b1* hepta-repeat. Amutant in *mediator of paramutation2 (mop2,* NRP(D/E)2a, 2nd largest subunit of Pol IV and V*)*, shows similar *b1*expression levels as *B'*, slightly increased repressive marks at the hepta-repeat, and a single-loop structure like *B'*. Mutants in *mop1* (RNA-dependent RNA polymerase) and *mop3* (NRPD1, largest subunit Pol IV) do show elevated *b1*expression, increased histone acetylation, and a multi-loop structure, similar to *B-I*. Furthermore, in line with a release of *B'* silencing, decreased H3K9me2 and H3K27me2 levels were observed in *mop1* and *mop3* mutants. Surprisingly, DNA methylation at the hepta-repeat is not significantly released in any of the mutants, indicating a role for the MOP1 RdRP and MOP3 NRPD1 in mediating H3K9me2 and H3K27me2 independent of their role in establishing DNA methylation.1. Hövel (2015) Sem. Cell & Dev. Biol. 44, 2. 2. Hollick (2017) Nat Rev. Genetics 18, 5. 3. Stam *et al.* (2002) Genes & Dev 16, 1906.

Gene / Gene Models described: *b1*; Booster grant (050-040-213) of the Netherlands Genomics Initiative; Systems Biology Research Priority fund of the University of Amsterdam

Funding acknowledgement: Booster grant (050-040-213) of the Netherlands Genomics Initiative; Systems Biology Research Priority fund of the University of Amsterdam

P229 @ClaireCodes

TIPs and tricks for identifying transposable element insertion polymorphisms in large genomes at the population-level

(submitted by Claire Menard <menar060@umn.edu>)
Full Author List: Menard, Claire C[1]; Catlin, Nathan S[3 4]; Platts, Adrian[3 4]; Munasinghe, Manisha[2]; Ou, Shujun[5]; Springer, Nathan[2]; Josephs, Emily B[3 4]; Hirsch, Candice N[1]
[1] Department of Agronomy and Plant Genetics, University of Minnesota, Minneapolis, MN, 55108, USA
[2] Department of Plant and Microbial Biology, University of Minnesota, Minneapolis, MN, 55108, USA
[3] Department of Plant Biology, Michigan State University, East Lansing, MI, 48824, USA
[4] Ecology, Evolution, and Behavior Program, Michigan State University, East Lansing, MI, 48824, USA
[5] Department of Molecular Genetics, The Ohio State University, Columbus, OH, 43210, USA

Transposable element insertion polymorphisms (TIPs) are TE sequences not found in the same location between individuals. Across many plant species, 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 to identify non-reference insertions. Now, with access to 700+ plant genome assemblies, and multiple assemblies within species, we are poised to study novel TE insertions at the genome and population levels. There are several methods that use short-read resequencing data for populations along with TE annotations for assembled genomes to accurately identify novel insertions in other individuals. These include TIP_finder, TEfinder, SPLITREADER and TEPID that were designed for use in small, lower complexity genomes. Here, we benchmark 8 programs, including a new tool we developed for highly repetitive genomes, using Arabidopsis and maize resequencing data to document metrics for accuracy and precision of TIPs identified by each program. We further characterize how these programs provide variable accuracy based on TE family, reference versus novel insertions, mappability of the region, TE age, and genomic context (genic vs non-genic regions). We also compare each program's runtime and memory efficiency. 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.

Funding acknowledgement: National Science Foundation (NSF)

P230

Targeted transposition in Arabidopsis

(submitted by Peng Liu <mcliupeng@gmail.com>)
Full Author List: Liu, Peng[1]; Edwards, Seth[1 2]; Pandesha, Pratheek[1 3]; Swanson, Ryan[1 2]; Hung, Yu-Hung[1]; Klaas, Gerald[1]; Panda, Kaushik[1]; Hancock, Nathan[4]; Slotkin, R.Keith[1 2]
[1] Donald Danforth Plant Science Center, Saint Louis, MO, USA 63146
[2] Division of Biological Sciences, University of Missouri-Columbia, Columbia, MO, USA 65211
[3] Department of Biology, Washington University in St. Louis, Saint Louis, MO, USA 63105
[4] University of South Carolina-Aiken, Aiken, SC, USA 29801

The placement of new DNA into specific locations in plant genomes is both inefficient and imprecise. This inefficiency hampers all approaches to develop new and enhance existing agricultural traits in crops. Technology such as CRISPR/Cas9 nucleases act like molecular “scissors” to cut DNA at specific locations in the genome, improving the precision of plant genome engineering. However, after cutting the DNA, the precise addition of new DNA has remained a challenge. This project identifies the missing counterpart to the molecular “scissors”--the molecular “glue” needed to precisely add DNA at sites cleaved by Cas9. We have used transposable elements to precisely add DNA into plant genomes, and tested the key rules governing this system of targeted DNA addition. We have successfully targeted the integration of new transposable element DNA into the genome of the discovery plant *Arabidopsis thaliana*. The overarching goal of this research is the production of a usable and accessible toolkit of technology that enables future plant synthetic biology approaches to introduce new custom DNA into plant genomes.

Funding acknowledgement: National Science Foundation (NSF), Bayer Crop Science, Donald Danforth Plant Science Center

P231
The impact of genic structural variants on gene expression profiles across the NAM founder lines

(submitted by Andrew Read <andycread@gmail.com>)
Full Author List: Read, Andrew C[1]; Munashinghe, Manisha[1]; Springer, Nathan[1]; Hirsch, Candice[1]
[1] University of Minnesota, Saint Paul, MN, 55108

Much of the genome-wide diversity within species is generated by the insertion and deletion of relatively large genomic sequences, often consisting of, or associated with, transposable elements. The resulting structural variation (SV) may change gene content or impact the expression profile of existing genes via introduction of regulatory elements and epigenetic changes. The high-quality genomic and transcriptomic datasets for the 26 maize NAM founder lines provides an opportunity to examine the impact of SVs on gene expression across diverse lines. We carried out pairwise whole genome alignments of B73 compared with each of the other 25 NAM genome assemblies to identify structural insertions and deletions of at least 50 bases. Genic SVs, defined as SVs that overlap gene coding sequence or the 1 kb promoter or downstream region, were included in our analysis. We find on average 5,000 genes with single SVs in the comparisons of any NAM genome with B73. Preliminary GO analysis indicates that genes involved in DNA and chromatin binding are enriched for the presence of SVs and that SV genes appear to be expressed at higher levels than genes without SVs. Analyses testing for associations between SV-presence/type and variability in gene expression patterns among tissues or genotypes will be presented.

Funding acknowledgement: National Science Foundation (NSF)

P232
Towards creating a comprehensive ARGONAUTE mutant collection in maize using CRISPR-Cas9

(submitted by Jason Lynn <jlynn@cshl.edu>)
Full Author List: Lynn, Jason S[1]; Martienssen, Robert A[2]
[1] Cold Spring Harbor Laboratory; Cold Spring Harbor, New York, USA 11724
[2] Howard Hughes Medical Institute; Cold Spring Harbor Laboratory; Cold Spring Harbor, New York, USA 11724

RNA interference (RNAi) is a broadly conserved mechanism that uses small RNAs to regulate gene expression and defend the cell against viruses and transposons. RNAi requires several steps: the biogenesis and processing of small RNAs, loading of small RNAs into an ARGONAUTE protein (AGO), and subsequent hybridization with target RNAs bearing sequence complementarity to the AGO-bound small RNA, leading to target cleavage and/or recruitment of other silencing factors. Plants have experienced an expansion of gene families encoding small RNA biogenesis/processing factors and Argonautes, correlating with increased function and dependency on gene silencing pathways in all aspects of plant physiology. For example, plant AGO2 is required for broad-spectrum resistance against viral infection by cleaving viral transcripts while closely related AGO4-clade proteins are required for RNA-directed DNA-methylation which suppresses transposition and maintains boundaries between active and silent chromatin. Plant AGOs exhibit dynamic expression, often being shuttled between cytoplasm and nucleus and are in some cases are phloem-mobile, allowing for rapid deployment of silencing throughout the plant. We have learned much about AGO since its discovery in *Arabidopsis thaliana* more than two decades ago, however, expansion of AGO gene families in maize and other crops suggests redundancy, specialization, and potential novel functions of RNAi that remain to be explored. To investigate the functional diversity of AGOs in maize, I used a multiplexed CRISPR-Cas9 approach to generate mutations in the highly-conserved catalytic PIWI domain for two different AGO genes per transgene construct. Using this approach for several AGOs, I've obtained new alleles for *Ago1a*, *Ago1c*, *Ago2a*, *Ago2b*, *Ago4a*, *Ago4b/Ago104*, *Ago5a*, and *Ago6*. Using this mutant collection, I will investigate the role of maize AGOs in paramutation, reproduction, and stress response. This new AGO mutant collection is a useful resource for characterizing the functions of RNAi pathways in maize and other crops.

Gene / Gene Models described: *ago1a,ago1c,ago2a,ago2b,ago4a,ago4b/ago104,ago5a/ago108,ago6*; Zm00001eb267430,Zm00001eb073810,Zm00001eb070760,Zm00001eb430920,Zm00001eb332830,Zm00001eb297760,Zm00001eb217290,Zm00001eb308470

Funding acknowledgement: National Science Foundation (NSF)

P233
Transposable elements as actors and sensors in the genome dynamics of maize and other flowering plants

(submitted by Jeff Bennetzen <maize@uga.edu>)
Full Author List: Bennetzen, Jeffrey L[1]; Frailey, Daniel C[1]; Giarratano, Rocco J[1]; Zhou, Hongye[1]
[1] Department of Genetics, University of Georgia, Athens, GA USA 30602

Along with polyploidy, transposable element (TE) action is the primary determinant of plant genome structure. After insertion, many plant transposable elements (especially the retroelements) lack the ability to excise, so they can be used to detect rates, types and outcomes of genome sequence variation in regions that are often selectively neutral. Comparison of the instability of genes to these "dead-on-arrival" (DOA) TEs can indicate the influence of selection on the mutations that persist in and near genes. Using PCR across pools of pollen grain, we were able to determine the rate of conversion of LTR (long terminal repeat) retrotransposons in maize into solo LTRs by homologous unequal recombination. This study also indicated the various factors that determine which LTR retrotransposons generate solo LTRs and which do not. By investigating DOA *Helitrons*, we were able to demonstrate the nature, mechanisms and astounding rates of maize genome change over the last few million years across 26 maize lineages. In addition, our *de novo* and consistent annotation of TE content across dozens of sequenced plant genomes indicated the patterns, or lack thereof, in the activation/amplification/silencing of all of the abundant TE families in each of these genomes and plant lineages.

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

P234 @SierraScientist
Blast from the Past: Maize breeding history and the rhizosphere microbiome

(submitted by Sierra Raglin <sraglin2@illinois.edu>)
Full Author List: Raglin, Sierra S.[1]; Ngumbi, Esther N.[2]; Kent, Angela D.[1]
[1] Department of Natural Resources and Environmental Sciences, University of Illinois at Urbana-Champaign, Urbana, IL, 61801
[2] Department of Entomology, University of Illinois at Urbana-Champaign, Urbana, IL, 61801

Maize (*Zea mays*) domestication and breeding drastically modified the morphology and physiology of modern germplasm, yet it is uncertain if the directed evolution of maize altered the diversity and complexity of the rhizosphere microbiome. However, as maize was adapted to North American agroecosystems, selection within highly managed environments mitigated pressures from abiotic and biotic stressors. Synthetic additives, such as nitrogen fertilizers and insecticides, became a management crutch now required for industrialized agroecosystems. Selection within these modified environments may have modulated the mechanisms of rhizosphere recruitment and filtration in modern germplasm. Therefore, we hypothesize that modern breeding within temperate industrial agroecosystems manipulated the structure of the rhizosphere microbiome. The goal of this research is to understand how domestication and northward migration of maize altered the diversity and structure of the rhizosphere microbiome. A chronosequence of maize spanning teosinte to modern inbred germplasm was grown in the greenhouse to sample the rhizosphere microbiome via bacterial 16S rRNA and fungal ITS amplicon sequencing. Multivariate statistics were used to identify breeding-induced changes in bacterial and fungal diversity and composition. Network analysis was used to identify breeding-driven changes in network organization, topology, and complexity. Ultimately this research serves as a stepping-stone to usher in a new era of germplasm development by leveraging the genetic diversity in wild and landrace relatives to understand the microbiome as an extended phenotype.

P235

Characterizing cis-regulatory evolution at scale across hundreds of wild grass species

(submitted by Charlie Hale <coh22@cornell.edu>)
Full Author List: Hale, Charles O[1]; AuBuchon-Elder, Taylor[4]; Casstevens, Terry[2]; Costa-Neto, Germano[2]; Hsu, Sheng-Kai[2]; Hufford, Matthew[5]; Johnson, Lynn[2]; Kellogg, Elizabeth A[4]; La, Thuy[2]; Miller, Zachary R[2]; Monier, Brandon[2]; Romay, M Cinta[2]; Schulz, Aimee J[1]; Seetharam, Arun[5]; Buckler, Edward S[1 2 3]
[1] Section of Plant Breeding and Genetics, Cornell University, Ithaca, NY USA 14853
[2] Institute for Genomic Diversity, Cornell University, Ithaca, NY USA 14853
[3] USDA-ARS; Ithaca, NY, USA 14853
[4] Donald Danforth Plant Science Center, St. Louis, MO USA 63132
[5] Iowa State University, Ames, IA USA 50011

Wild plant genomes are becoming increasingly available, providing plant breeders the opportunity to access significantly more genetic variation than previously possible. To utilize this variation, methods to scale functional genomic analyses across many species are now needed, particularly for identifying important non-coding variation. We investigated cis-regulatory evolution in a panel of globally-distributed Andropogoneae grass species, which were collected and used to generate dozens of chromosome-level assemblies and hundreds of additional whole genome assemblies from short reads. We leveraged these assemblies to quantify occurrence rates of transcription factor (TF) binding motifs in putative regulatory regions, an approach that can measure expanding or contracting regulatory networks. We hypothesize that for some TFs, occurrence of motifs is associated with adaptation to divergent temperature regimes across the evolutionary history of grasses. After controlling for evolutionary relatedness, genomic background and genome quality, we built models associating motif occurrence rates with environmental variables. Our approach provides a scalable method to identify TFs that may have been central to adaptation over macroevolutionary time scales, nominating candidates for functional validation.

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

P236

Convergent adaptation of maize and sorghum to soils with low phosphorus availability.

(submitted by Nirwan Tandukar <ntanduk@ncsu.edu>)
Full Author List: Tandukar, Nirwan[1 2]; Zapata, Fausto R[1 2]; Alvarez, Ruben R[2]; Tzeng, Jung-Ying[3]
[1] Department of Genetics and Genomics, Thomas Hall, North Carolina State University, Raleigh, 27607
[2] Department of Molecular and Structural Biochemistry, Polk Hall, North Carolina State University, Raleigh, 27607
[3] Department of Statistics and Bioinformatics Research Center. Ricks Hall, North Carolina State University, Raleigh, 27607

Environmental variables such as nutrient availability exert evolutionary constraints that select for genetic variables, leading to better adaptation to local environment conditions. When species adapt to similar environmental conditions, selection on similar genes can indicate repeated or convergent mechanisms of adaptation. We have previously identified the role of High PhosphatidylCholine 1 (hpc1) as a phosphatidylcholine modulator, using lipid variation in Mexican highland maize adapted to low phosphorus and cold. We are now using high-dimensional genetic datasets of Sorghum bicolor from Africa and Zea mays from the Americas to perform environmental GWASs and FST measurements for different phenotypes relating to phosphorus availability, concentration, and utilization. Comprehensive research on the matter is laborious, costly, and time extensive due to the overwhelming number of genes and their regulatory networks. Thus, it is necessary to develop a robust statistical framework that can combine individual p-values, aggregating multiple small effects and redefining the order of emphasis. Here, we use the Generalized Berk Jones (GBJ) statistic for gene set analysis which accounts for linkage disequilibrium among SNPs in a set. Preliminary analysis in Sorghum has identified SORBI_3003G038000, an ortholog to hpc1, as the candidate with the most hits for phosphorus GWASs. Pathway-level GBJ analysis has also shown “Phosphatidylcholine acyl editing” as one of the top hits for biological processes.

Funding acknowledgement: Department of Energy (DOE)

P237

Detection of abnormal chromosome 10 in genotype-by-sequencing data

(submitted by Meghan Brady <meghan.brady@uga.edu>)
Full Author List: Brady, Meghan J[1]; Dawe, R. Kelly[1 2]
[1] University of Georgia - Dept. of Genetics; Athens, GA, US 30602
[2] University of Georgia - Dept. of Plant Biology; Athens, GA, US 30602

Abnormal chromosome 10 (Ab10) is a larger variant of normal chromosome 10 (N10) that acts as a female meiotic driver. Ab10 alters the frequency of alleles linked to special loci (knobs) found throughout the genome and thus has had a significant affect on the evolution of Maize (Zea mays). The largest previous study of Ab10 distribution surveyed 140 Maize landraces and 20 Teosinte populations using PCR markers and florescence in situ hybridization (FISH). In this study, I seek to develop a high throughput method to detect Ab10 in order to facilitate larger surveys of its distribution. Using the wealth of publicly available genotype-by-sequencing (GBS) data for Maize landraces and Teosinte, I demonstrate that it is possible to detect Ab10 presence absence and differentiate two of the structural variants of Ab10. I detected 469 previously un-sequenced Ab10 isolates representing a ~4000% increase. I found that 8% of Maize landrace and 12% of Teosinte individuals contain Ab10. In the future, I will use MaxENT to develop an environmental niche model for Ab10.

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

P238

Genetic characterization and population structure of North American popcorn

(submitted by Madsen Sullivan <madsens2@illinois.edu>)
Full Author List: Sullivan, Madsen[1]; Williams II, Martin M[2]; Studer, Anthony J[1]
[1] Department of Crop Sciences, University of Illinois, Urbana, IL 61801, USA.
[2] Global Change and Photosynthesis Research Unit, USDA-ARS, Urbana, IL 61801, USA.

Popcorn is an important crop in the United States; however, genetic analyses of popcorn are limited and tend to utilize relatively few markers that likely underestimate the total genomic variation. A panel of 362 popcorn accessions of North American, Latin American, and global origins was evaluated using 417,218 single nucleotide polymorphisms generated using a genotyping-by-sequencing approach. Using this genomic data, a model-based clustering analysis using the ADMIXTURE software identified two groups of popcorn within the panel. The first group was composed of North American Yellow Pearl Popcorns and several accessions of the Chilean Curagua landrace. The second group was designated as Pointed and Latin American Popcorns, as it included all remaining North American accessions (pointed and early popcorns), Latin American accessions, and all accessions of global origin. These two populations exhibited large differences in linkage disequilibrium decay, frequency of monomorphic sites, and minor allele frequencies. Specifically, the North American Yellow Pearl Popcorns showed characteristics of a highly inbred population with limited genetic diversity compared to the Pointed and Latin American Popcorns. Interestingly, the Pointed and Latin American Popcorns were shown to be closely related to seventeen teosinte inbred lines, more so than sweet corn or dent corn lines. Furthermore, phenotypic differences were observed, as North American Yellow Pearl Popcorns generally had yellow kernels and tolerance to nicosulfuron, while approximately half of the Pointed and Latin American Popcorns had white kernels and a higher incidence of nicosulfuron injury. A filtered marker set was curated and used for genome-wide association studies to investigate the genetic architecture of these traits in popcorn. Finally, these analyses identified popcorn-specific candidate genes for nicosulfuron tolerance. This genomic characterization will enable popcorn breeding programs to accelerate the rate of genetic gain and identify sources of genetic diversity to incorporate into elite popcorn germplasm.

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

P239

Genetic dissection of kernel protein concentration using near-isogenic lines derived from the Illinois long term selection experiment containing the FLOURY2-RFP reporter

(submitted by Catherine Li <chli6@illinois.edu>)
Full Author List: Li, Catherine H[1]; Arp, Jennifer J[1]; Heller, Nicholas[2]; Moose, Stephen P[1]
[1] Department of Crop Sciences, University of Illinois Urbana-Champaign, Urbana, IL 61801, USA
[2] Department of Agriculture, Illinois State University, Normal, IL, 61761, USA

The Illinois Long Term Selection Experiment is the longest-running continuous genetics experiment in higher plants. More than 350 cycles of artificial selection performed over 120 years have generated distinct populations representing the phenotypic extremes of kernel protein concentration in maize. To help unravel regions of the maize genome that contributed to the rapid and dramatic responses to phenotypic selection, we have recently derived four populations of near-isogenic lines from combinations of the Illinois Protein Strains. After 6 generations of backcrossing, which included phenotypic selection for the "opposite" kernel protein concentration of the recurrent parent (e.g. higher protein in the Illinois Low Protein background), the populations visibly resemble their recurrent parents. Kernel protein concentration was measured using both near-infrared reflectance and pink color intensity of the FLOURY2-RFP reporter transgene, an easily visualized and non-destructive marker for alpha-zeins, whose accumulation varies dramatically among the Illinois Protein Strains. One conclusion from these near-isogenic lines is that not all introgressions differed from the recurrent parent, thus not all genomic regions contribute to phenotypic responses to selection. Previous genotype-by-sequencing analyses of the Illinois Protein Strains identified 306 SNPs whose patterns of diversity showed strong correlations with both forward and reverse selection for grain protein. Near-isogenic line pairs for two regions harboring some of these SNPs and candidate genes for asparagine cycling, another pathway strongly affected by selection, displayed phenotypic changes that validate their contribution to grain protein concentration. Updated analyses using the version 5 reference genome and genotyping of these near-isogenic lines will aid in narrowing down the list of candidate genes causing the dramatic variation in kernel protein concentration.

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

P240

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

(submitted by Heather Chamberlain-Irwin <hchamber@iastate.edu>)
Full Author List: Chamberlain-Irwin, Heather N.[1]; Somerville, Andrew D.[2]; Hufford, Matthew B.[1]
[1] Department of Ecology, Evolution, & Organismal Biology, Iowa State University, Ames, IA 50011, USA
[2] World Languages and Cultures, Iowa State University, Ames, IA 50011, USA

What can ancient maize DNA tell us about social and political change? As human populations have shifted, so have local preferences for domesticated crops. To fully understand the evolution of maize, we must therefore consider its relationship with people. Our project utilizes comparative genomics of ancient and modern maize to understand evolution in an area with 3000 years of documented maize cultivation: Moquegua Valley, Peru. This fertile river valley in the northern Atacama Desert was initially home to subsistence farmers, the Huaracane, who utilized floodplain agriculture. The region was later colonized by the first governed society, or state, in South America, the Tiwanaku. Tiwanaku arose in the Andean highlands (3800 m.a.s.l.) and increased maize consumption as its population grew and embraced the use of maize beer, chicha, to elicit labor and form political alliances. Tiwanaku people migrated to the Moquegua Valley, installed irrigation canals, and maize cultivation intensified. They likely leveraged pre-existing trade routes to the fertile Moquegua Valley to import maize to the highland city. These social and political changes may have brought about biological changes in maize, namely through potential shifts from locally adapted lowland varieties to the Tiwanaku people's preferred highland varieties. Admixture between these maize populations may have facilitated adaptation of preferred varieties in this new ecological context. The relationship between the Tiwanaku and Huaracane societies may have left genetic legacies in maize in the Moquegua Valley that extend to the present day. We are currently assessing these dynamics by establishing a historical transect of ancient and modern maize samples from the region for genetic analysis.

P241 @ArunSeetharam

Genomes of 35 representative species of Andropogoneae Clade provide insight into the evolution, diversification, and adaptation of major crops

(submitted by Arun Seetharam <arnstrm@iastate.edu>)
Full Author List: Seetharam, Arun S[1]; Schulz, Aimee J[2]; Sitzer, Michelle[2]; Scheben, Armin[3]; Aubuchon-Elder, Taylor[4]; Bukowski, Robert[2]; Fengler, Kevin[5]; Kremling, Karl A[6]; La, Thuy M[2]; Lepak, Nick K[7]; Llaca, Victor[5]; Phillips, Alyssa R[8]; Portwood, John L[9]; Romay, M. Cinta[2]; Song, Baoxing[10]; Sun, Qi[2]; Syring, Michael L[1]; Woodhouse, Margaret R[9]; Siepel, Adam C[3]; Ross-Ibarra, Jeffrey[8]; Buckler, Edward S[2]; Kellogg, Elizabeth A[4]; Hufford, Matthew B[1]
[1] Iowa State University, Ames, Iowa, USA 50014
[2] Cornell University, Ithaca, NY, USA 14853
[3] Cold Spring Harbor Laboratory, NY, USA 11724
[4] Donald Danforth Plant Science Center, Olivette, MO, USA 63132
[5] Corteva Agriscience, Johnston, IA, USA 50131
[6] Inari Agriculture, Cambridge, MA, USA 02139
[7] Plant, Soil and Nutrition Research, Agricultural Research Service, United States Department of Agriculture, Ithaca, NY 14853
[8] University of California, Davis, CA, USA 95616
[9] Corn Insects and Crop Genetics Research Unit, Agricultural Research Service, United States Department of Agriculture, IA 50011
[10] Peking University, Haidian District, Beijing, China 100871

The grass family encompasses more than 10,000 species making it the fourth-largest family in the plant kingdom. Among those, Maize, sorghum, and sugarcane are widely cultivated and are considered the most productive and water-efficient crops and biofuels in the world. They belong to the Andropogoneae tribe of grasses, which contains a thousand species that collectively represent over a billion years of evolutionary history. To understand the diversity and evolution across this tribe, we sampled 35 species worldwide, covering a wide spectrum of genetic diversity in Andropogoneae. The scaffold/chromosomal level genome assemblies were constructed using the PacBio HiFi or CLR data and were scaffolded using BioNano optical maps. For the Zea genus, the pseudomolecule assemblies were constructed using pangenome anchor markers (genetic map). The evidence-based annotations were inferred using the RNA-seq data from various tissues and using homology to known gene models of existing assemblies. The relative abundance and composition of various repeat families were characterized using the EDTA package. All genomes are made available via MaizeGDB on the Toronto agreement. Further analyses are underway using modern genomics and machine learning to survey and analyze these related species, determining the most important genetic features they share that allow them to adapt to heat and drought across the Andropogoneae tribe.

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

P242

How does a maize relative withstand deep freezing temperatures?

(submitted by Elad Oren <eo235@cornell.edu>)
Full Author List: Oren, Elad[1]; Zhai, Jingjing[1]; Rooney, Travis E[2]; Angelovici, Ruthie[3]; La, Thuy[1]; Lepak, Nick[4]; Romay, Cinta[1]; Buckler, Edward S[1 4]
[1] Institute for Genomic Diversity, Cornell University, Ithaca, NY 14853, USA
[2] Sesaco Corporation, Austin, TX 78701, USA
[3] Division of Biological Sciences and Interdisciplinary Plant Group, University of Missouri, Columbia, Missouri 65211, USA
[4] USDA-ARS, Robert W. Holley Center for Agriculture and Health, Ithaca, NY 14853, USA

By extending the growing season of annual crops, net primary productivity and yield can be improved, and the mismatch between soil nitrate availability and crop nitrate demand can be addressed. Early maize planting requires cold tolerance in temperate regions, which is not present in current elite maize. *Tripsacum dactyloides*, the closest perennial and cold-tolerant relative of maize, can be used to identify genes related to cold adaptation. To this end, we crossed *Tripsacum* accessions collected from high- and low latitude habitats and screened the hybrids in a field trial in Ithaca, NY over two years, where the winter reaches deep freezing temperatures. We analyzed amino acid composition and protein expression levels from each sample of the hybrids' rhizomes and roots taken in winter (dormant stage) and in summer (active stage), using a shotgun proteomics approach. We identified 4,465 proteins with high quality data, of which 120 are differentially expressed between summer and winter. Our current candidate set focuses on 30 proteins that are at least 5× more abundant in winter than in summer. These include lipid transfer proteins, heat shock proteins, late embryogenesis abundant (LEA) proteins, and other drought-related proteins. We are using genomic data available from ~40 related *Andropogoneae* grass species to further study candidate gene families and the regulatory evolution of these candidate proteins, and we expect to nominate candidate genes for improving cold tolerance in modern maize.

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

P243 @snodgrasshopper

Identifying fractionation events across the *Tripsacinae* subtribe

(submitted by Samantha Snodgrass <snodgras@iastate.edu>)
Full Author List: Snodgrass, Samantha J.[1]; Seetharam, Arun[1 2]; Woodhouse, Margaret R.[3]; Hufford, Matthew B.[1]
[1] Ecology Evolution and Organismal Biology Dept., Iowa State University, Ames, IA, USA, 50010
[2] Genetics Development and Cellular Biology Dept., Iowa State University, Ames, IA, USA, 50010
[3] USDA-ARS, Corn Insects and Crop Genetics Research Unit, Ames, IA, USA, 50010

Polyploidy and fractionation-- the processes by which genomes grow and shrink in size-- characterize plant genome evolution. The ancestral species of the *Tripsacinae* subtribe, which contains the genera *Tripsacum* and *Zea*, underwent a whole genome duplication event (WGD) ~12MYA after divergence from *Sorghum*. The descendant species that comprise the subtribe, including *Zea mays* ssp. *mays*, have since fractionated back to a diploid-like state and, in some cases (ex. *Zea perennis*), have undergone another round of polyploidy. With high-quality, *de novo* assemblies of species from across both genera, we can compare shared and differential fractionation between lineages sharing the same WGD event. First, we must accurately call fractionation events across multiple genomes. Previous work identifying fractionation events has relied heavily on manual curation due to issues with automated pipelines. This becomes impractical when scaling to larger numbers of genomes. Thus, we have created a new pipeline that includes more sensitive whole genome alignment (AnchorWave) and identifies orthologous homeolog pairs across multiple genomes (GENESPACE, pSONIC). Together, this allows us to call small-scale deletion events (GATK), which identifies shared and divergent fractionation events across lineages. This scalable pipeline will ease comparisons of divergent genomes and begin to address remaining questions surrounding fractionation, both within the *Tripsacinae* and more broadly, as a complex and dynamic process of genome evolution.

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

P244
Identifying structural variation across diverse *Zea* genomes

(submitted by Elena Jiang-Grieser <elenaj@iastate.edu>)
Full Author List: Jiang, Elena[1]; Seetharam, Arun[1]; Hufford, Matthew[1]
[1] Iowa State University, Ames, IA, 50010

Structural variants (SVs) are a broad class of polymorphism that have received increasing attention with the growing catalog of sequenced maize genomes. These are often characterized at a lower size limit of 100 base pairs (bp), extending up to several million bp. SVs are typically categorized into four main groups: large insertions and deletions, inversions, duplications, and translocations. In many cases, SVs have been linked to phenotypic variation relevant to an organism's fitness and they are known to play an important role in both adaptation and speciation. We are leveraging *de novo* genome assemblies across the maize NAM (Nested Association Mapping) founder lines and teosinte to confidently assess structural variation at a fine scale in tropical and temperate as well as wild and domesticated germplasm.Whole genome alignment tools such as AnchorWave have allowed us to overcome the limitations of previous local alignment pipelines, particularly when dealing with TE-rich genomic regions, large inversions, and large indels often found in maize genomes. We have paired our Anchorwave and local alignments with several new pipelines to detect SVs to gauge their relative strengths (AnchorWave, MUMmer4+SyRI, MUM&Co, Minimap2 + SyRI). The optimal pipeline will detect SVs with higher precision and sensitivity. Once finalized, we will utilize our pipeline to evaluate population structure in our diverse germplasm and putative selection on SVs during maize domestication and adaptation.

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

P245 @MohamedElWalid
Mapping freezing tolerance in tripsacum by bulk segregant analysis

(submitted by Mohamed El-Walid <mze3@cornell.edu>)
Full Author List: El-Walid, Mohamed Z[1]; Hsu, Sheng-Kai[2]; Seetharam, Arun[3]; Gault, Christy[2]; Costich, Denise E.[2]; Romay, M Cinta[2]; Hufford, Matthew B.[3]; Lepak, Nick[4]; La, Thuy[2]; Buckler, Edward S.[1 2 4]
[1] School of Integrative Plant Sciences, Section of Plant Breeding and Genetics, Cornell University, Ithaca, NY USA 14853
[2] Institute for Genomic Diversity, Cornell University, Ithaca, NY USA 14853
[3] Department of Ecology, Evolution, and Organismal Biology, Iowa State University, Ames, IA USA, 50011
[4] USDA-ARS; Ithaca, NY, USA 14853

Increased variability in weather patterns is one of many consequences of climate change. Agronomic approaches including shifting planting zones or planting earlier to avoid excessive heat during flowering can address these challenges. However, these solutions result in an increased likelihood of crops to encounter freezing events. For these reasons, it is imperative to improve freezing/chilling tolerance in maize to maintain and improve plant productivity. Tripsacum dactyloides, a close relative of maize that diverged about 650 thousand years ago, can be found across the Americas. As a perennial, this species must withstand elongated freezing temperatures in northern latitudes. The genetic similarity between Tripsacum and maize may allow for freezing tolerance to be transferred to maize. To identify causal freezing tolerance genes, we used a bulk segregant analysis (BSA) to find QTL within a diverse Tripsacum population. An initial founder population of 304 geographically distinct clones was collected from across the US and used to generate an F1 population from crosses between northern and southern clones. F2 seed was then generated for use in freezing screens where seedlings were subjected to -7°C overnight temperatures. Illumina short-read sequencing was performed on the founders, F1 population and F2 freezing tolerant and susceptible bulks. The diversity and structure of this population allows the contributions of founders to be observed at the chromosome scale as well as site-by-site resolution through testing alleles for significant segregation between bulks. To identify founder contributions to each bulk, Pac-Bio Hifi genomes have been generated for two founders to be used as reference genomes. Preliminary results have shown founders contribute at varying rates across the genome between bulks and have highlighted candidate loci associated with freezing tolerance.

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

P246 @cryan4plants

Molecular evolution of a reproductive barrier across twelve million years

(submitted by Elli Cryan <epcryan@ucdavis.edu>)
Full Author List: Cryan, Elli P[1 2]; Phinney, A Garnet[2]; Seetharam, Arun S[3 4]; Kliebenstein, Daniel J[1]; Ross-Ibarra, Jeffrey[2]
[1] Plant Sciences; UC Davis; Davis, CA, USA 95616
[2] Evolution and Ecology; UC Davis; Davis, CA, USA 95616
[3] Ecology, Evolution and Organismal Biology; Iowa State University; Ames, IA, USA 50011
[4] Genetics, Development and Cell Biology; Iowa State University; Ames, IA, USA 50011

In maize and teosinte, three complex loci encode gametophytic factors 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 between individuals from different populations. However, this barrier is not complete. Infrequent fertilization of incompatible gametes facilitates introgression of gametophytic factor genes into other populations. Previous modeling shows that as the frequency of functional pollen factors increases in outside populations, the benefit of the prezygotic barrier degrades, and any adaptive benefit of preventing outside fertilization is transient. This suggests 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 in many genomes, including: modern maize lines, *Zea mays* teosinte subspecies, other members of the *Zea* genus and *Tripsacinae* subtribe, and species as diverged as *Sorghum bicolor*. Across these genomes, genes in the three gametophytic factor 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 the NAM parent lines, identify candidate orthologous genes in related species, construct gene trees of known and candidate functional genes, and analyze rates of molecular evolution. The male and female factors have very similar sequences across all three loci, so we define orthologous groups by genomic synteny. We find evidence of potentially functional gametophytic factors in our sampled genomes, including in lineages that have been estimated to be twelve million years diverged. By documenting the genetic diversity and history of this prezygotic barrier system within and across species, we hope to better understand how a theoretically transient system has persisted through time.

Funding acknowledgement: National Science Foundation (NSF), UC Davis Plant Sciences

P247

Phylogenetic variation of potential nitrification rate in rhizosphere of diverse grass species in Andropogoneae

(submitted by Sheng-Kai Hsu <sh2246@cornell.edu>)
Full Author List: Hsu, Sheng-Kai[1]; Emmett, Bryan[2]; Haffke, Alexandria[2]; La, Thuy[1]; Lepak, Nick[3]; AuBuchon-Elder, Taylor[4]; Kellogg, Elizabeth A[4]; Buckler, Edward S[1 3]
[1] Institute for Genomic Diversity, Cornell University, Ithaca, NY, USA 14853
[2] USDA-ARS, National Laboratory for Agriculture and the Environment, Ames, IA, USA 50011
[3] USDA-ARS, Robert W. Holley Center for Agriculture and Health, Ithaca, NY, USA 14853
[4] Donald Danforth Plant Science Center, St. Louis, MO USA 63132

Nitrogen fertilization in modern crop production has a profound impact on the nitrogen cycle in the ecosystem. Not only economically costly, excess nitrogen in the soil leads to nitrous oxide emissions, a potent greenhouse gas, and to nitrate leaching into groundwater. Breeding for efficient nitrogen use and recycling in a cropping system is crucial for global sustainability. An optimized strategy for nitrogen reuse may not exist in modern crops given the lack of selection and strong bottleneck during domestication. However, solutions may be present in their wild relatives. Hence, we studied the nitrogen cycle in the rhizosphere of ~40 grass species related to maize and sorghum. We discovered significant genetic variation in potential nitrification rates of the rhizosphere soil of diverse species, evidencing the capability of grass species to affect a key step of the soil nitrogen cycle. In addition, we plan to measure the nitrogen loss from the rhizosphere soil system to evaluate the efficiency of nitrogen use and recycling more directly. With genomic and environmental data collected for the studied species, we aim to tackle the genetic basis and ecological relevance of the phylogenetic variation in nitrogen use and recycling. Our goal is to nominate and introduce key genes and pathways used across wild grass species to maintain a more sustainable nitrogen cycle in maize breeding programs.

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

P248 @Michael00857273

The Hordeum genus: A rich Resource for adaptive genetic variation

(submitted by Michael Anokye <anomic17@gmail.com>)
Full Author List: Anokye, Michael[1]; Westhoff, Philipp[2]; Kopriva, Stanislav[3]; Schmising, Maria von Korff[1]
[1] Institute of Plant Genetics, Heinrich-Heine-Universität, Düsseldorf, Nordrhein-Westfalen, 40225, Germany
[2] CEPLAS Metabolomics Laboratory , Heinrich-Heine-Universität, Düsseldorf, Nordrhein-Westfalen, 40225, Germany
[3] Institute for Plant Sciences, University of Cologne, Cologne, Nordrhein-Westfalen, 50674, Germany

Annual crops including wheat, barley, rice and maize represent majority of world's food supply. Annual agriculture has however been questioned in terms of sustainability for our ecosystem. Apart from tilling the field every growing season, fields lie fallow and are exposed to erosion, weed killers and fertilizers have to be applied so that the seeds can sprout and grow. Contrary, perennial plants live for many years, develop deep roots that protect the soil from erosion and access low-lying water and nutrient resources. The wild relatives of our annual plants are often perennial and thus present a valuable resource for breeding perennial grains for stress environments. Using diverse grass genus Hordeum originating from arid, and temperate environments of South-North America, and Eurasia, we investigated to what extent differences in life-history strategies are connected to nutrient resource capture and use efficiencies, shoot growth dynamics and reproduction. We trialled 43 annual-perennial Hordeum species including barley under outdoor conditions at University of Düsseldorf, Germany between 2021 and 2022 growing seasons. Our data suggest that annuals were fast in growth and development reflected in significant build-up of aboveground biomass after 45d of field establishment and reaching anthesis earlier than perennials. Annuals accumulated significant levels of essential plant mineral elements including nitrogen, phosphorus and sulfur measured in the flag leaf at grain filling compared to perennials. Despite slow growth and developments, perennials produced longer spike with increased floret number at grain filling. We found modest correlation between flag leaf element composition and yield related traits such as TGW within perennials. Annuals however, displayed marked tendency of remobilizing acquired nutrient elements to support grain development. Taken together, perennials compromised grain productivity for vegetative growth with extended duration of flowering. Generating hybrids of crosses between selected annuals and perennials could be an interesting area of future work.

Funding acknowledgement: PERLIFE, ERC

P249

The *Ga1* locus of the genus *Zea* is associated with genome structures derived from multiple, independent non-homologous recombination events

(submitted by Amruta Bapat <amruta03@iastate.edu>)
Full Author List: Bapat, Amruta R[1 2]; Moran Lauter, Adrienne N.[3]; Hufford, Matthew B.[4]; Boerman, Nicholas A.[2]; Scott, Paul M.[3]
[1] Interdepartmental Genetics and Genomics Program, Iowa State University, Ames, Iowa, USA 50011
[2] Department of Agronomy, Iowa State University, Ames, Iowa, USA 50011
[3] USDA-ARS, Corn Insects and Crop Genetics Research Unit, Ames, Iowa, USA 50011
[4] Department of Ecology and Evolutionary Biology, Iowa State University, Ames, Iowa, USA 50011

The maize *Ga1* locus controls cross incompatibility between field corn and popcorn varieties. The *Ga1-S* haplotype mediates its effect through pollen- and silk-specific factors that interact genetically to enable correct pollen tube growth and fertilization. This haplotype contains two pectin methylesterase (PME) genes, *ZmPme3* and *ZmGa1P* that are expressed in silk and pollen, respectively. While the *Ga1-S* haplotype occurring in most popcorn has several copies of *ZmGa1P*, repeat sequences derived from both *ZmPme3* and *ZmGa1P* have been observed in lines lacking the haplotype. This haplotype known as *ga1* is widely present in field corn. The primary objective of this study is to characterize these repeat sequences from a diverse collection of maize and teosinte to use this information to better understand the evolution of the *Ga1* locus. First, we examined the complexity of the region at this locus in all field corn and popcorn lines for which high-quality maize genome assemblies are currently available. DNA sequence analysis of genome region of *Ga1* locus led to the categorization of the genomes into 5 groups based on the number and type of PME-like sequences found at this region. Second, we studied the duplication events that led to the tandemly repeated sequence arrays. Phylogenetic reconstruction using a maximum-likelihood approach was used to infer a duplication history for the *ga1* and *Ga1-S* repeat regions. The *ga1* phylogeny suggests that at least two separate duplication events occurred very close to each other in time, while the *Ga1-S* phylogeny suggests a series of duplications that occurred during the evolution of the locus. Divergence estimates of the duplicates of the *ga1* haplotype time the event to more than 600 kya whereas those in *Ga1-S* to three time periods i.e. > 600 kya, ~ 260 kya and ~ 100 kya. These estimates suggest that the *ga1* and *Ga1-S* duplication events occurred independently of each other, however, both likely involved a non-homologous recombination mechanism, suggesting that this region of the genome may be a hot-spot for non-homologous recombination events. Finally, we also identified and studied *ZmPme3* and *ZmGa1P* homologs in *Zea* and *Tripsacum* genomes. The results suggest that *ga1* and *Ga1-S* repeats originated from an ancestral pair of PME genes that duplicated and diverged in ancestral *Zea* genomes. It is likely that these loci then introgressed in *Zea mays* from *non-mays Zea* genomes. In conclusion, the *Ga1* locus is a hotspot for tandem duplication events and presents an important case study that may provide insights into the evolution of repeated regions of genomes.

Funding acknowledgement: United States Department of Agriculture (USDA)

P250

A Mendelian genetic model to explain the non-Mendelian segregation ratios of UND-9

(submitted by Chris Larson <christopher.d.larson@und.edu>)
Full Author List: Larson, Christopher D[1]; Brunelle, Dale C[1]; Clark, Janice K[1]; Sheridan, William F[1]
[1] University of North Dakota; Grand Forks, North Dakota, USA 58202

The UND-9 mutant allele in maize is an embryo-specific mutation obtained by EMS treatment of W22 pollen. The UND-9 mutant was crossed onto the inbred B73 and during the following and subsequent generations of crossing onto B73 the mutant allele behaved in a non-Mendelian fashion. Under the normal model as a recessive, single gene allele, it is expected that the embryos in the kernels of a self-pollinated ear on a plant heterozygous for this allele would segregate at around 25% for the mutant embryo phenotype. However, this is not the case, with such trials regularly producing segregation ratios of mutant embryos much higher or lower than this at frequencies far beyond what random chance would produce. We've grown and examined multiple lineages of this mutant, with the allele exhibiting a broad range of segregation values over three generations. This has resulted in 440 self-pollinated ears of maize for our analysis. Our analysis of hereditary patterns has allowed us to confirm that transmission of the mutant allele through both the male and female gametophyte occurs normally. Here, we propose a genetic model in which UND-9 follows a similar method of regulation to that of *striate2* and its repressor gene *Inhibitor of striate*, with the UND-9 mutant phenotype being repressed by repressor genes obtained by the original and subsequent crosses to B73. We hypothesize that the independent assortment of three dominant repressor alleles inherited from B73 in the self-pollinated plants heterozygous for UND-9 can explain the wide variety of segregation ratios we see among the progeny of the self-pollinated plants. We are further working to map and sequence the gene of interest, with the possibility that this information may lead us to the repressor genes as well.

P251

A Novel mutational approach to uncover genetic determinants of hybrid vigor in maize

(submitted by Emily Kuhn <kuhn43@purdue.edu>)
Full Author List: Kuhn, Emily A[1]; Li, Ying[1]; Johal, Gurmukh[1]
[1] Purdue University; West Lafayette, IN 47907

Heterosis, or hybrid vigor, is a phenomenon observed in both plant and animal systems where hybrid offspring perform better when compared to their parents. For hybrid plants, this can result in increased biomass, crop yields, and vigor when compared to the inbred parents. Even though heterosis has been used in agriculture for over a century, the molecular mechanisms that result in hybrid vigor remain elusive even after years of investigation. A molecular understanding of heterosis is desirable because it will speed up the process of breeding compatible inbred lines for developing hybrid seeds and it will provide us with the knowledge to potentially engineer inbred lines that can mimic the beneficial phenotypic effects of heterosis, eliminating the need for farmers to buy new hybrid seeds every year. The goal of this research project is to identify genes that are required for the heterotic phenotypes seen in hybrid maize. The working hypothesis is that a mutation in genes that are essential for heterosis will cause an altered heterotic phenotype in hybrid maize plants. To determine these genes, we apply a forward genetics approach to identify mutant hybrids with altered heterosis, and then detect causal genes via whole genome sequencing. We will also integrate the whole genome sequencing analysis with hybrid transcriptome datasets to prioritize candidate genes that are important for heterosis.

Funding acknowledgement: Purdue AgSEED grant

P252

A maize protoplast assay provides insight into the regulation of terpene synthase 23, a gene involved in plant defense against Western Corn Rootworm

(submitted by Janik Telleria Marloth <janik.telleria-marloth@pharmazie.uni-halle.de>)
Full Author List: Telleria Marloth, Janik[1]; Bley, Sebastian[1]; Schaff, Claudia[1]; Degenhardt, Jörg[1]
[1] Martin Luther University Halle, Institute for Pharmacy, Hoher Weg 8, Halle, Germany, D- 06120

In many maize lines, attack of the Western corn rootworm *Diabrotica virgifera virgifera* induces the release of the sesquiterpene (*E*)-β-caryophyllene. This volatile is known for its versatile function in defense against herbivory, but the molecular mechanism behind the herbivory-related induction remains unclear. To study this mechanism, we focused on the regulation of terpene synthase TPS23 which is responsible for the biosynthesis of (*E*)-β-caryophyllene. The promoter of *TPS23* from the maize line *Delprim* was used to conduct yeast one-hybrid screens. These screens resulted in a variety of interacting candidate genes. After filtering the candidate genes for transcription factors (TFs), the rescued plasmids were re-transformed into the constructed *TPS23* promoter:reporter strain to further eliminate false positives. Some of the candidate plasmids contained only parts of the coding sequence. Therefore, the full length sequences were isolated and cloned into yeast expression vectors to re-examine the interactions between the promoter and TFs. To exclude the effects of the heterologous gene regulation environment of yeast, we established a homologous expression system consisting of B73 mesophyll protoplasts from etiolated seedlings.
The combination of a promoter::*eGFP* construct and a mCherry fusion protein construct was used to test the interaction of the TFs by fluorescence. To study the role of TFs within the induction mechanism, the transformed protoplasts were incubated with jasmonic acid, an important stress hormone involved in plant defense after herbivory. Here, we present the activation of the *TPS23* promoter in protoplast by jasmonic acid and in response to co-expression of some TFs.

Gene / Gene Models described: *TPS23*; Zm00001d024234

P253

A multidisciplinary approach to assess the roles of *Glossy2* and *Glossy2-like* in maize cuticular wax biosynthesis

(submitted by Dirk Winkelman <dwink@iastate.edu>)
Full Author List: Winkelman, Dirk C[1 4]; Alexander, Liza E[1 4]; Trost, Elysse[2 5]; Lopez, Miriam[3]; Lauter, Nick[3]; Nikolau, Basil J[1 4]; Yandeau-Nelson, Marna D[2 4]
[1] Roy J. Carver Department of Biochemistry, Biophysics and Molecular Biology, Iowa State University, Ames, IA 50011
[2] Department of Genetics, Development and Cell Biology, Iowa State University, Ames, IA 50011
[3] USDA-ARS Corn Insects and Crop Genetics Research Unit, Ames, IA 50011
[4] Center for Metabolic Biology, Iowa State University, Ames, IA 50011
[5] Undergraduate Genetics Major, Iowa State University, Ames, IA 50011

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 are detrimental to plant health. The cuticle is composed of a network of solvent-extractable cuticular waxes that are both intercalated within and laid atop an insoluble cutin polyester matrix. Depending on the organ and stage of development, the cuticular waxes are comprised of combinations of different lipids, namely 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 is providing insights on cuticle formation. This study focuses on the maize *Glossy2* (*Gl2*) gene, which encodes a protein that is archetypal for the BAHD class of acyltransferases. 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 maize fatty acid elongase complex (FAE). The recently identified GL2-paralog, GLOSSY2-LIKE, shares 63% amino acid similarity with GL2. To assess the *in planta* physiological function of *Gl2-like,* six unique maize mutant alleles were generated via CRISPR-Cas9 genome editing. Cuticle analyses of different organ types in single *gl2* and *gl2-like* mutants, as well as *gl2;gl2-like* double mutants, demonstrate both overlapping and distinct functions of these genes. The functionality of GL2 and GL2-LIKE proteins is being investigated via a synthetic biology approach in yeast, in which each paralog is being expressed in an engineered strain in which the entire maize FAE system has been reconstructed. This combination of multidisciplinary strategies is unraveling the roles that *Gl2* and *Gl2-like* serve in maize cuticle biosynthesis.

Funding acknowledgement: National Science Foundation (NSF)

P254 @SinghaDNA

A novel DNAJ-thioredoxin-like protein impacts carbohydrate partitioning in maize

(submitted by Singha Dhungana <srdm93@umsystem.edu>)
Full Author List: Dhungana, Singha R.[1]; McCubbin, Tyler[2]; Baker, R. Frank[1]; Braun, David M.[1 2]
[1] Division of Biological Sciences, Interdisciplinary Plant Group, and Missouri Maize Center, University of Missouri-Columbia, Columbia, MO 65211
[2] Division of Plant Science and Technology, Interdisciplinary Plant Group, and Missouri Maize Center, University of Missouri-Columbia, Columbia, MO 65211

Carbohydrate partitioning is the process by which sugars, primarily sucrose, synthesized in the photosynthetic tissues, such as mature leaves, are mobilized to non-photosynthetic tissues, such as roots, seeds, and developing organs. As the carbohydrates produced by plants are the primary source of energy for most lifeforms on earth, understanding how these compounds are allocated to different plant tissues is crucial. Significant progress has been made in understanding the physiological, anatomical, and biochemical processes governing carbohydrate partitioning, but the underlying genetics is still poorly characterized. With the aim of exploring this process, we identified two allelic recessive mutants from EMS mutagenized populations*, carbohydrate partitioning defective13 (cpd13)* and *cpd35*. Both mutants exhibit carbohydrate partitioning defects, including reduced plant growth, chlorotic leaves, and hyperaccumulation of soluble sugars and starch in mature leaves. Interestingly, the mature mutant leaves display a unique crossbanding pattern of chlorotic and green regions and occasionally exude sugary droplets. We mapped the causative mutations to a gene encoding a protein containing DNAJ-like and thioredoxin-like domains by using fine mapping and whole genome sequencing-based approaches. While proteins containing both of these domains are not well studied in plants, some DNAJ-like proteins have protein-folding activity and are known to be involved in protein quality control in other organisms. In tobacco leaves, translational fusions of CPD13-red fluorescent protein localized to the endoplasmic reticulum (ER). Furthermore, the chlorotic phenotype was induced when mutant plants were grown under high temperature and high light. We hypothesize that the failure to export sugar in the mutant leaves is due to the inability of the defective CPD13 protein to properly interact with or process target proteins. Ongoing studies will help explore this hypothesis and elucidate the function of this novel protein in carbohydrate partitioning and the basis of the peculiar crossbanding leaf phenotype.

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

P255

A shared maize biochemical defense pathway enzyme contributes to plant growth

(submitted by Eric Schmelz <eschmelz@ucsd.edu>)
Full Author List: Ding, Yezhang[1 2]; Khalil, Ahmed S.[1]; Salib, Mariam N.[3]; Poretsky, Elly[1]; Murphy, Katherine M.[4]; Tarkowská, Danuše[5]; Nian Char, Si[6]; Yang, Bing[4 6]; Molinski, Tadeusz F.[3 7]; Zerbe, Philipp[8]; Huffaker, Alisa[1]; Schmelz, Eric A.[1]
[1] Department of Cell and Developmental Biology, University of California at San Diego, La Jolla, CA USA
[2] Lawrence Berkeley National Laboratory, Berkeley, CA 94720, USA (current address)
[3] Department of Chemistry and Biochemistry, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093, USA
[4] Donald Danforth Plant Science Center, 975 N Warson Road, St. Louis, MO, USA
[5] Laboratory of Growth Regulators, The Czech Academy of Sciences, Institute of Experimental Botany & Palacký University, Šlechtitelů 27, CZ-78371 Olomouc, Czech Republic
[6] Division of Plant Sciences, Bond Life Sciences Center, University of Missouri, Columbia, MO, USA
[7] Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093, USA
[8] Department of Plant Biology, University of California Davis, One Shields Avenue, Davis, CA, USA

Plant stress protection is partly mediated by diverse specialized metabolites derived from duplicated pathway genes underlying primary metabolism and hormone biosynthesis. Continued shared use of conserved precursors requires multiple levels of regulation to balance growth and defense; however, examples of impactful pathway exchanges remain uncommon. Maize growth requires the *ent*-copalyl diphosphate (*ent*-CPP) synthase (Anther ear 1: ZmAn1) which drives gibberellin (GA) biosynthesis, while defense relies on two microbially-regulated diterpenoid pathways, namely kauralexins and dolabralexins, which require a separate shared *ent*-CPP synthase, termed Anther ear 2 (ZmAn2). Defense pathway branches requiring *ent*-CPP precursors are further specified by two kaurene synthase like (KSL) enzymes, namely ZmKSL2 and ZmKLS4, separate from those involved in GA biosynthesis. To better understand the scale of defense metabolism, we identified nor (19C)- and dinor (18C)-diterpenoids as dominant dolabralexin end-products in field grown roots displaying genetic associations with the gene encoding a cytochrome P450 enzyme, ZmCYP71Z16. In heterologous enzyme expression systems, ZmCYP71Z16 was required to reproducibly generate the diverse carbon-carbon bond cleavage products discovered. To block abundant production of both dolabralexins and kauralexins, we created a *Zmksl2kls4* double mutant deficient in both defense pathways. Notably, *Zmksl2kls4* mutant plants maintain ZmAn2-mediated *ent*-CPP accumulation which could still serve as a GA biosynthetic precursor. Under controlled growth conditions, defense-impaired *Zmksl2kls4* mutant plants display significantly greater levels of *ent*-copalol, bioactive GAs and 2.5-fold increases in biomass compared to respective wild type plants. Our findings are consistent with the ability of defense pathway precursors to significantly contribute to GA biosynthesis and plant growth. Biochemical and genetic efforts to understand maize specialized metabolism powerfully reveal how dynamic and complex plant-biotic interactions can influence plant growth phenotypes.

Gene / Gene Models described: *An2, KSL2, KSL4, CYP71Z16*; Zm00001d029648, Zm00001d041082, Zm00001d032858, Zm00001d014136
Funding acknowledgement: National Science Foundation (NSF), United States Department of Agriculture (USDA)

P256

Analysis of phytochemical accumulation and antioxidant activity during maturation of corn grains containing both carotenoids and anthocyanins

(submitted by Hwan-Hee Bae <miami9@korea.kr>)
Full Author List: Bae, Hwan-Hee[1]; Ha, Jun Young[1]; Go, Young Sam[1]; Son, Jae-Han[1]; Kang, Kyeong Min[1]; Son, Beom-Young[1]; Jung, Tae-Wook[1]
[1] Department of Central Area Crop Science, National Institute of Crop Science, Rural Development Administration, Suwon, 16429, Republic of Korea

Corn (*Zea mays* L.) contains phytochemicals such as carotenoids, anthocyanins, phlobaphenes, and phenolics which provide many health benefits. The pigment accumulation of carotenoids and flavonoids, which generate yellow to deep orange pigments, and flavonoids, including phlobaphenes and anthocyanins, which produce red, blue, and purple pigments, determine kernel color. To improve corn's health benefits, we developed a single-cross hybrid corn, 'Hwanggeumheukchal' that accumulates both yellow and purple pigments. In this study, we investigated the patterns in phytochemical accumulation and antioxidant activity while filling daily grain samples of fresh corn 'Hwanggeumheukchal'. Anthocyanin contents began to accumulate in grains 15 days after pollination (DAP) and increased steadily during grain filling. Carotenoid levels were highest at 10288.0±521.7 μg g^{-1} in 18 DAP and decreased throughout grain maturity. The stage of total flavonoids and polyphenol levels that were the most abundant were 19 DAP (1033.7±86.1 μg of CE g^{-1}) and 30 DAP (3430.8±252.0 μg of GAE g^{-1}), respectively. The scavenging capacity for 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radicals gradually increased, with variations depending on the time. However, reducing capacity of 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) didn't change significantly in the range of 1185.7-1226.6 μg of TE g^{-1} over the grain maturity stage. Anthocyanin (-0.855; *p*

Funding acknowledgement: Rural Development Administration (RDA)

P257

Causes and consequences of endogenous hypoxia on growth and metabolism of the developing maize kernel

(submitted by Matthias Langer <langer@ipk-gatersleben.de>)
Full Author List: Langer, Matthias[1]; Hilo, Alexander[1]; Guan, Jiahn-Chou[2]; Koch, Karen[2]; Gündel, Andre[1]; Wagner, Steffen[1]; Ortleb, Stefan[1]; Radchuk, Volodymyr[1]; Mayer, Simon[1]; Borisjuk, Ljudmilla[1]; Rolletschek, Hardy[1]
[1] Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Seeland-Gatersleben, Germany
[2] University of Florida, Horticultural Sciences Department, Gainesville, FL, USA

Maize kernels are the largest cereal grains and their endosperm is severely oxygen deficient during grain fill. The causes, dynamics, and mechanisms of acclimation to hypoxia are minimally understood. We demonstrate here that hypoxia develops in the small, growing endosperm, but not the nucellus, and becomes the standard state regardless of diverse structural and genetic perturbations in modern maize (B73, popcorn, sweet corn), mutants (*sweet4c, glossy6, waxy*), and non-domesticated wild relatives (teosintes and *Tripsacum* species). Magnetic resonance imaging (MRI), enabling high resolution imaging of the seed interior, identified moisture gradients inside endosperm, having relevance for oxygen diffusivity. By combining MRI with infrared microspectroscopy, we also uncovered lipidous layers, surrounding the endosperm and potentially hampering oxygen diffusion. Manipulation of oxygen supply induced reciprocal shifts in gene expression implicated in control of mitochondrial functions (23.6 kDa HSP, VDAC2) and multiple signalling pathways (core hypoxia genes, cyclic nucleotide metabolism, ethylene synthesis). Metabolite profiling revealed oxygen-dependent shifts in mitochondrial pathways, ascorbate metabolism, starch synthesis, and auxin degradation. Elevated oxygen supply over long-term enhanced the rate of kernel development but reduced dry matter accumulation. Altogether, evidence here supports a mechanistic framework for establishment of, and acclimation to hypoxia in the maize endosperm.

Funding acknowledgement: Deutsche Forschungsgemeinschaft

P258

Characterization of candidate genes related to cuticular wax deposition on maize silks

(submitted by Madison Lane <mlane@iastate.edu>)
Full Author List: Lane, Madison[1 2]; Chen, Keting[1]; Guo, Yuxiang[1 3]; Yandeau-Nelson, Marna[1 2 3]
[1] Department of Genetics, Development and Cell Biology, Iowa State University, Ames, IA 50011
[2] Molecular, Cellular and Developmental Biology Interdepartmental Graduate Program
[3] Bioinformatics and Computational Biology Interdepartmental Graduate Program

The hydrophobic cuticle, which covers aerial portions of plants, is the first line of defense against environmental stresses, including drought, UV radiation, temperature, and insects and pathogens. This cuticle is comprised of a cutin polyester matrix that is infused with and laid atop by cuticular waxes, comprised of differing combinations of very long chain fatty acids (VLCFAs), hydrocarbons, aldehydes, alcohols, esters, and ketones. The cuticle on maize silks is rich in hydrocarbons, with minor amounts of VLCFAs and trace aldehydes and alcohols that together provide important protection for this tissue during the pollination period. Cuticle biosynthesis within the epidermal cells is tightly regulated at both the transcriptional and post-transcriptional levels. Many of the transcription factors known to regulate cuticle biosynthesis and deposition in Arabidopsis and in maize are not expressed in silks, suggesting different transcriptional regulation in this important organ. We have identified two transcription factors through multi-omics approaches that are putatively related to cuticle composition in either silks or seedlings (i.e. FDL1 and a bZIP TF). In addition, we have identified genes involved in the fatty acid elongation pathway (e.g. a Ketoacyl-CoA Synthetase) and in lipid transport (e.g. lipid transfer proteins that may facilitate wax transport through the cell wall) that are putatively associated with cuticular wax composition on maize silks. The potential functions of these candidate genes in regulation of cuticle biosynthesis and deposition on silks have been assessed by profiling cuticular waxes on silks from UniformMu mutants of these candidate genes. Characterization of these genes will allow a better understanding of how the plant cuticle is formed and deposited, and lays the foundation for future applied breeding approaches to generate "designer" protective cuticles.

Gene / Gene Models described: *FDL1, NSLTPLIKE,TGA6,KCS6*; Zm00001d022227, Zm00001d029365, Zm00001d010658, Zm00001d028241
Funding acknowledgement: National Science Foundation (NSF)

P259

Characterization of seedling growth and embryonic root systems of *carbohydrate partitioning defective* mutants in maize

(submitted by Jason Roberts <jtrgmc@umsystem.edu>)
Full Author List: Roberts, Jason T.[1]; McCubbin, Tyler[2]; Braun, David M.[1 2]
[1] Division of Biological Sciences, Interdisciplinary Plant Group, and Missouri Maize Center, University of Missouri-Columbia, Columbia, MO 65211
[2] Division of Plant Science and Technology, Interdisciplinary Plant Group, and Missouri Maize Center, University of Missouri-Columbia, Columbia, MO 65211

Carbohydrate partitioning is the process by which sugars synthesized in photosynthetic tissue (leaves) are transported to non-photosynthetic tissue (such as roots). Plants that exhibit inhibition of efficient carbohydrate transport are termed *carbohydrate partitioning defective* (*cpd)* mutants. While the leaves of these mutant plants are known to be affected little is understood about how the developing root systems are affected in the mutant. Recently, we have tested two *cpd* mutants (*Cpd1* and *cpd7*) for early developmental root phenotypes. By using a newly implemented sterile media plate assay protocol, followed by a measurement of their primary roots and shoots lengths using an image processing software (ImageJ), we made several interesting discoveries. *Cpd1* individuals do not exhibit an early developmental phenotype in their primary root and shoot, while *cpd7* individuals display a shorter primary root and shoot in comparison to its wild-type siblings. With this knowledge, we can direct our studies to formulate hypotheses based on these results. For instance, we are exploring *cpd48,* another mutant allele of *cpd7,* using the same approach to determine the phenotypic outcome of *cpd48* mutant individuals.
The identification of early developmental root phenotypes across various *cpd* mutants will open possibilities for further investigations into the gene's functions. Moreover, with the new plate assay protocol, we can direct our research into testing the impacts of various exogenous sugars and substances to observe their effect on maize's embryonic root system.

Funding acknowledgement: National Science Foundation (NSF), A&S Undergraduate Research Mentorship Program

P260

Combinatorial effects of the ACT-like domain and small molecule on the regulatory activity of the maize bHLH transcription factor R1

(submitted by Nan Jiang <jiangn11@msu.edu>)
Full Author List: Jiang, Nan[1]; Lee, Yun Sun[1]; Morohashi, Kengo[1]; Tacderas, Audrey[1]; Kuehn, Spencer[1]; Grotewold, Erich[1]
[1] Department of Biochemistry and Molecular Biology, Michigan State University, East Lansing, MI 48823

In the maize aleurone, the basic helix-loop-helix (bHLH) transcription factor R interacts with the R2R3-MYB regulator C1 to activate the anthocyanin biosynthesis pathway. However, it is unclear how the coordinate regulation of all the pathway genes is accomplished, without any obvious conservation of the respective regulatory regions. Our previous studies showed that the monomer/dimer configuration of an ACT-like domain at the C-terminus of R affects the DNA-binding activity of the R bHLH motif. We proposed a model in which when the ACT-like domain forms a dimer, the bHLH is monomeric and R is tethered to DNA indirectly, through the interaction with C1. When the dimerization of the ACT-like is impaired, then the bHLH motif dimerizes and recognizes a canonical G-box (CACGTG). To further elucidate the mechanisms by which the ACT-like domain affects DNA-binding by the bHLH motif, we examined the *in vitro* DNA binding capacity and kinetics of the bHLH motif on the promoter region of anthocyanin biosynthetic gene *A1* and canonical G-box in the absence/presence of the ACT-like domain. Using amplified luminescent proximity homogeneous assay (ALPHA), we identified non-canonical DNA-binding sites of the bHLH motif in the *A1* promoter and demonstrated that, in the presence of the ACT-like domain, the binding of bHLH to the *A1* promoter or canonical G-box shows similar affinity (K_D) but different kinetics (K_{on} and K_{off} rates). Preliminary results indicated small molecules, including flavonoid pathway intermediates, contribute to the formation of different transcriptional complexes through interactions with the ACT-like domain. Results will be discussed in the context of what this means for the regulation of the anthocyanin pathway, and for how ACT-like domains present in ~30% of the plant bHLH transcription factors influence gene regulation. Funding for this project was provided by grants from the National Science Foundation MCB-1822343 and IOS-1733633.

Gene / Gene Models described: *r1 - colored1*; Zm00001eb429330
Funding acknowledgement: National Science Foundation (NSF)

P261

Comparing phenolic compound accumulation in maize and sorghum

(submitted by Leah Durst <durstlea@msu.edu>)
Full Author List: Durst, Leah[1 2]; Gomez-Cano, Lina[2]; Sitar, Sidney[1]; Newton, Linsey[1]; Grotewold, Erich[2]; Thompson, Addie[1]
[1] Department of Plant, Soil & Microbial Sciences; Michigan State University; East Lansing, MI 48824
[2] Department of Biochemistry and Molecular Biology; Michigan State University; East Lansing, MI 48824

Phenolic compounds are found in all plants and have a variety of different uses within the plant. They function as antioxidants, produce an antifungal response, protect the plant from ultra violet rays, and act in many other ways. Previous work has investigated compound accumulation in healthy maize seedlings and in maize infected with the fungus that causes tar spot disease. This study seeks to quantify phenolic compounds in sorghum and to compare the range of variation between species. Though it is known that maize and healthy sorghum seedlings produce some of the same phenolic compounds, the range of natural accumulation and the compounds unique to each species were unknown. Through methanol extraction of phenolic compounds in sorghum seedlings and liquid chromatography - mass spectrometry (LC-MS) readings, we investigated the accumulation of over 20 different phenolic compounds in sorghum and compared quantities between the two species using existing maize data. Most of the phenolic compound accumulations varied drastically between species, but the accumulation of sinapic acid and vanillic acid were very similar. The differences in accumulation between species reflect differences in genetic content or expression in the biochemical pathways that produce these compounds. This preliminary research will be used for further study of the biochemical pathways in both species, and could have implications for bioenergetic research in sorghum.

Funding acknowledgement: United States Department of Agriculture (USDA)

P262

Developing an efficient gene-editing method for tropical maize protoplasts using CRISPR/Cas9

(submitted by Lauren Higa <higalaur@hawaii.edu>)
Full Author List: Higa, Lauren[1]; Blank, Max[1]; Uehara, Ashlyn[1]; Hampson, Ella[2]; Lee, Keunsub[3]; Muszynski, Michael[2]; Du, Zhi-Yan[1]
[1] Department of Molecular Biosciences and Bioengineering, University of Hawaii at Manoa, Honolulu, Hawaii 96822, USA
[2] Department of Tropical Plant and Soil Sciences, University of Hawaii at Manoa, Honolulu, Hawaii 96822, USA
[3] Department of Agronomy, Iowa State University, Ames, Iowa 50011, USA

As the rising global population and the effects of climate change threaten food security worldwide, researchers are turning to genome editing technology to improve agronomic traits of crops, such as maize. Maize is one of the most produced crops globally and is a staple crop for many regions in the tropics, where climate-induced stresses are worsening. The emergence of the clustered regulatory interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9) system has revolutionized genome editing with its notable accuracy, efficiency, and simplicity. It opens new opportunities for researchers to develop more productive and stress-resilient maize varieties. Protoplast transfection offers a quick and reliable approach to validating the genome editing reagents of the CRISPR/Cas9 system prior to the transfection of embryos. The system described here resulted in over 30% transfection efficiency and viable cells that lasted up to 7 days.

Gene / Gene Models described: *gl2*; Zm00001eb071110
Funding acknowledgement: National Science Foundation (NSF)

P263

Development of multi-functional CRISPR system in fast-flowering mini maize to study proteomic rebalancing and other complex metabolic traits

(submitted by Huda Ansaf <hah34@umsystem.edu>)
Full Author List: Ansaf, Huda B.[1]; Yang, Bing[2]; Holding, David R.[3]; Birchler, James A.[1]; Angelovici, Ruthie[1]
[1] University of Missouri; Division of Biological Sciences, Columbia, Missouri, 65211
[2] University of Missouri; Division of Plant Science and Technology, Columbia, Missouri, 65211
[3] University of Nebraska; Department of Agronomy and Horticulture, Lincoln, Nebraska, 68588

Amino acids are proteins' building blocks, making them extremely important for growth and development. Crop seeds, such as legumes and cereals, play an essential role as a key food source in the diet of humans and livestock but do not meet the dietary requirements of essential amino acids (EAA). Lacking sufficient levels of EAA in the diet can lead to protein-energy malnutrition, adversely affecting the immune, gastrointestinal, nervous, and cardiovascular systems. Efforts to fortify AA composition in crop seeds have had minimal success since plants respond to induced protein composition alterations by activating a conserved and phenotypically robust regulatory mechanism that “resets” it to its original state. Proteomic rebalancing, like many other complex metabolic traits, is driven by multiple gene networks, and therefore its perturbation will require the manipulation of multiple genes in parallel. Hence, this project aims to develop a powerful high throughput multifunctional genome editing approach in FFMM to make it an ideal system for accelerated biofortification efforts. This system can lead to automation and high throughput scan of candidate genes ensuing from quantitative genomic-based approaches in a tissue-specific manner, which will highly benefit the field of genetic engineering. The creation of a multifunctional CRISPR-Cas system will enable accelerated discoveries to increase the biological and nutritional values of major cereal crops.

P264

Engineered 6-phosphogluconate dehydrogenase in amyloplasts assessed in field corn hybrids for mitigation of grain yield loss under heat stress

(submitted by Hope Hersh <hopehersh@ufl.edu>)
Full Author List: Hersh, Hope L[1 2]; Ribeiro, Camila[3]; Hannah, Curtis L.[1 2]; Settles, Mark A.[1 2 4]
[1] University of Florida, Plant Molecular and Cellular Biology Program, Gainesville, Florida, United States 32601
[2] University of Florida, Horticultural Sciences Department; Gainesville, Florida, United States 32601
[3] Brazilian Agricultural Research Corporation, Embrapa Maize & Sorghum, Rodovia MG-424, Km 45, Sete Lagoas- MG- Brazil 35701-970
[4] National Aeronautics and Space Administration, Ames Research Center, De France Ave, Mountain View, California, United States 94043

Heat stress reduces maize grain weight and quality. Starch synthesis in the endosperm is sensitive to heat stress and potentially is a limiting pathway for grain yield under heat stress. In addition to enzymes involved in starch biosynthesis, chloroplast-localized 6-phosphogluconate dehydrogenase (PGD3) is critical for starch accumulation. PGD3 is one of three enzymes in the oxidative portion of the pentose phosphate pathway. Maize encodes two cytosolic isozymes, PGD1 and PGD2. These isozymes are heat stable, while amyloplast-localized PGD3 is heat labile under in vitro and in vivo heat stress conditions. A heat stable 6-phosphogluconate dehydrogenase localized to amyloplast was previously developed by fusing the waxy1 N-terminal plastid targeting sequence to the Pgd1 and Pgd2 open reading frames. Previous work showed that WPGD1 and WPGD2 transgenes complement the pgd3 defective kernel phenotype suggesting the fusion proteins are targeted to the amyloplast. Initial field trials suggest that WPGD1 and WPGD2 mitigate part of the yield losses due to heat stress. We generated B73 x W22 *Wpgd* hybrids to assess transgenic and non-transgenic plants in a field trial comparing heat-stressed and non-stressed planting dates. Heat stressed plots showed yield losses and analyses of the hybrid transgenic plots show mitigation of these yield losses.

Funding acknowledgement: United States Department of Agriculture (USDA)

P265 @RuthieLucille

Fine tune regulation of flowering time in maize via phospholipid interaction with ZCN8

(submitted by Ruthie Stokes <rstokes@ncsu.edu>)
Full Author List: Stokes, Ruthie L[1]; Barnes, Allison C[1]; Thompson, Peter M[1]; Rellán-Álvarez, Rubén[1]
[1] North Carolina State University; Raleigh, NC, USA 27703

Maize was originally domesticated from its wild relative teosinte parviglumis in southwestern Mexico around 9,000 years ago. After domestication, maize expanded into higher latitudes in North America, where it adapted to longer days. Many genes were involved in photoperiod sensitivity adaptation, including Zea Centroradialis 8 (ZCN8), which is a mobile florigen gene. ZCN8 underlies a flowering time QTL in American maize populations. Variants in the promoter of ZCN8, selected in high-latitude maize, lead to higher expression of ZCN8 and are associated with shorter flowering time1. ZCN8 is a homolog of the protein Flowering Time (FT) in Arabidopsis. In Arabidopsis FT is capable of binding to phosphatidylcholine (PC) and phosphatidylglycerol (PG)2 to modulate flowering time. Using heterologous expression, we recently showed that ZCN8 co-purifies with certain PC species, particularly abundant in high-elevation Mexican maize varieties that carry a non-functional allele of High Phosphatidylcholine 1 (HPC1)3.We also showed that this HPC1 allele is associated with shorter flowering times and higher fitness in highland conditions. Here, we seek to understand the mechanisms by which phospholipid interaction with ZCN8 may regulate flowering time using heterologous protein expression platformsWe show that ZCN8 produced in Saccharomyces is bound to PC 34:2 and generate maize protoplasts to explore lipid-protein interactions in native membrane environments.. Using comparative and predictive modeling, I will then show the predicted binding sites of ZCN8 modeled after it's FT homolog.In the future, I will try to crystalize ZCN8, to use the structure to formally identify lipid binding sites and study enzymatic activity. This will expound upon our knowledge of how phospholipid-protein interactions can mediate flowering time.References 1)Guo, Li et al. Current Biology 2018. 2)Meng, Xin et al. Plant Cell 2011. 3)Barnes, Allison et al. PNAS. 2022.

Gene / Gene Models described: *ZCN8, HPC1*; Zm00001e353250, Zm00001e121780
Funding acknowledgement: National Science Foundation (NSF)

P266

Functional analysis of High PhosphatidylCholine 1 (hpc1): A phospholipase involved in maize adaptation to high elevations

(submitted by Hannah Pil <hdpil@ncsu.edu>)
Full Author List: Pil, Hannah D[1]; Barnes, Allison C[1]; Rellán Álvarez, Rubén[1]
[1] Department of Molecular and Structural Biochemistry, North Carolina State University, Raleigh, NC, USA

Despite its cultivation for thousands of years and across varying climates, the genetic and molecular basis of the adaptation of maize is far from fully understood. We previously found Zea mays to have observable phospholipid variation across maize accessions adapted to different elevations. This is largely attributed to the gene High PhosphatidylCholine 1 (hpc1), which encodes a phospholipase A1 enzyme (Barnes, Rodríguez-Zapata, Juárez-Nuñez et al. 2021). Our data indicates impaired function of the highland allele of this gene found in Palomero Toluqueño maize (hpc1-PT) when compared to the lowland inbred line B73. Maize varieties carrying the highland allele have shorter flowering times and higher fitness when grown in their local highland environment but not in lowland environments. However, the exact mechanisms of action of hpc1 on survival and reproductive success remain largely unknown.To further investigate hpc1, we will use CRISPR-CAS9 maize mutants of hpc1 in addition to mutants of Arabidopsis homologs DAD1-Like Lipase 3 (DALL3) and DAD1-Like Lipase 4 (DALL4) for heterologous expression. Preliminary data suggests that both these maize and Arabidopsis mutants flower earlier in cold conditions than the controls, phenocopying hpc1-PT. These mutant lines will be assessed against their respective wild-types for lipid content, flowering time, cold tolerance, and effects of low phosphate. Additionally, we plan to complement the Arabidopsis mutants to explore the impact of maize hpc1 alleles hpc1-PT and hpc1-B73 on their lipid profiles and fitness, aiming to assess the potential effect of maize hpc1 on a different plant-based system. With these studies, we hope to elucidate the functional differences of hpc1 alleles in maize adaptation to low temperature and phosphorus deficiency.

Gene / Gene Models described: *hpc1, DALL3, DALL4*; Zm00001eb121780, AT2G30550, AT1G06800
Funding acknowledgement: National Science Foundation (NSF), Department of Energy (DOE)

P267

Functional characterization of genes in the maize fatty acid elongation pathway using synthetic biology approaches in yeast

(submitted by Elysse Trost <eltrost@iastate.edu>)
Full Author List: Trost, Elysse L.[1]; Winkelman, Dirk C.[2]; Alexander, Liza E.[2]; Stenback, Kenna[2]; Nikolau, Basil J.[2]; Yandeau-Nelson, Marna D.[3]
[1] Undergraduate Genetics Major, College of Liberal Arts and Sciences; Ames, IA, United States 50011
[2] Department of Biochemistry, Biophysics, and Molecular Biology; Ames, IA, United States 50011
[3] Department of Genetics, Development, and Cell Biology; Ames, IA, United States 50011

The plant cuticle is a hydrophobic barrier that is the first line of defense between aerial organs and the external environment. The cuticular waxes protect against both biological and non-biological stressors and are composed of fatty acids and their derivatives. The very long chain fatty acyl-CoAs that are precursors for cuticular wax biosynthesis are synthesized by the fatty acid elongation pathway in maize. The maize *Ketoacyl-CoA Synthetase (KCS)* and *Glossy2* gene families are two of many enzyme families that are necessary for the elongation of the very long chain fatty acyl-CoA precursors. Based on previous research, specific genes within these two families may interact either directly or indirectly to affect the elongation of fatty acids: *KCS5* and/or *KCS6* with *Glossy2 (Gl2)* and/or *Glossy2-like (Gl2-lk)*. Moreover, the role of the *Glossy2* gene family in maize fatty acid elongation is not understood. To study the relationship between the *KCS* and *Glossy2* gene families, we took a synthetic biology approach by expressing and characterizing different combinations of these maize genes in yeast. Expression of either *Gl2* or *Gl2-lk* negatively impacted yeast growth in combination with some *KCSs*, as shown by increased doubling times. Very long chain fatty acid (VLCFA) production in the different *KCS-Gl2* engineered strains grown to stationary phase exhibited some differences in profiles, particularly in fatty acids with acyl-chain lengths of C24 and above. These differences in fatty acid production will next be explored in engineered yeast strains expressing all maize fatty acid elongation pathway genes. This synthetic biology approach is enabling the functional analysis of the *Glossy2* gene family and how these genes may differentially interact with the genetically diverse maize fatty acid elongation pathway.

Funding acknowledgement: National Science Foundation (NSF)

P268

GWAS identification of genes associated with phenolic metabolism

(submitted by Lina Gomez Cano <gomezca5@msu.edu>)
Full Author List: Gomez Cano, Lina[1]; Rodriguez, Jonas[2]; de Leon, Natalia[2]; Grotewold, Erich[1]
[1] Department of Biochemistry and Molecular Biology, Michigan State University, East Lansing, MI 48824, USA
[2] Department of Agronomy, University of Wisconsin-Madison, Madison, WI 53706, USA

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 stressful 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 identify genes associated with the formation and regulation of phenolic compounds in maize by conducting 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 a number of 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. This research is supported by the National Science Foundation under Grant No. 1733633. LGC is supported by a Fellowship from Michigan State University under the Training Program in Plant Biotechnology for Health and Sustainability (T32-GM110523). JR is supported by a UW-Madison SciMed GRS Fellowship.

Funding acknowledgement: National Institutes of Health (NIH)

P269 @_Nandan_Rai

Genetic variation in NRT1.1b contributes to improved nitrogen utilization in maize

(submitted by Maruti Nandan Rai <mnrai@illinois.edu>)
Full Author List: Rai, Maruti Nandan[1 2]; Rhodes, Brian[1]; Ross, Edward Ross[1]; Moose, Stephen[1 2]
[1] Department of Crop Sciences, University of Illinois at Urbana Champaign, IL, USA
[2] Carl R. Woese Institute for Genomic Biology, University of Illinois at Urbana-Champaign, IL, USA

Nitrogen utilization, defined as the ratio of grain yield to accumulated plant nitrogen, is an important target for enhancing the economic and environmental sustainability of maize production. Genetic mapping of nitrogen utilization in a hybrid population detected many QTL. For the strongest effect QTL, allelic variation in gene structure and expression coupled with patterns of genetic diversity indicated *Nitrate Transporter 1.1b (NRT1.1b)* as a primary candidate gene. The *NRT1.1* gene family is highly conserved among plants and variation in *NRT1.1b* has been shown to contribute to the higher NUE of indica compared to japonica rice populations. Multiplex CRISPR-Cas9 mutagenesis targeting the maize *NRT1.1* gene family in the H99 inbred background generated knockout alleles of both *NRT1.1b* and *NRT1.1a*, due to single-base frameshift mutations. When grown in replicated trials at our nitrogen-responsive field site, both as inbred lines and as hybrids following crosses with multiple testers, these mutations alter nitrogen utilization phenotypes. The *nrt1.1b* mutant exhibits stronger phenotypes that are consistent with its role in both nitrate transport and signaling. We found that the B73 haplotype for *NRT1.1b* is expressed nearly 10-fold lower than the Mo17 haplotype, likely due to a transposon insertion in the B73 proximal promoter. Interestingly, when hybridized with B73, *NRT1.1B* RNA expression is reduced to the level of the B73 haplotype. Surveys of maize diversity panel show that the B73 haplotype is rare within the 282-association panel but has increased 10-fold in ex-PVP germplasm. Collectively, our results suggest that weakened expression of *NRT1.1B* in maize hybrids attenuates feedback inhibition of N uptake, thereby promoting enhanced nitrogen utilization. The dominant suppressor action of the *NRT1.1B* haplotype in B73, the most prominent founder of the Stiff Stalk heterotic group, suggests selection at *NRT1.1B* has contributed to recent breeding improvements for nitrogen utilization among U.S. Corn Belt hybrids.

Gene / Gene Models described: *NRT1.1b*; GRMZM2G161459
Funding acknowledgement: Department of Energy (DOE)

P270

Heterologous expression in *Nicotiana benthamiana* identifies candidate effector proteins from *Phyllachora maydis* that suppress cell surface-triggered immune responses

(submitted by Namrata Jaiswal <namrata.jaiswal@usda.gov>)
Full Author List: Jaiswal, Namrata[1]; Rogers, Abigail[2]; MacCready, Joshua S[3]; Chilvers, Martin[3]; Scofield, Steven R[1]; Iyer-Pascuzzi, Anjali[2]; Helm, Matthew[1]
[1] Crop Production and Pest Control Research Unit, U.S. Department of Agriculture-Agricultural Research Service (USDA-ARS), West Lafayette, IN 47907, U.S.A.
[2] Department of Botany and Plant Pathology, Purdue University West Lafayette, IN 47907, U.S.A.
[3] Department of Plant, Soil, and Microbial Sciences, Michigan State University, East Lansing, MI, 48824

Fungal pathogens often secrete virulence (effector) proteins into plant cells during the infection process to modulate host immune responses. Recent genomic and transcriptomic studies of *Phyllachora maydis*, an ascomycete pathogen that causes tar spot disease in maize, revealed this fungal pathogen encodes 163 putative effector proteins, of which eighteen are abundantly expressed during disease development. Here, we used heterologous expression in *Nicotiana benthamiana* to elucidate whether any of the eighteen candidate effector proteins from *P. maydis* have effector-like functions. Live-cell imaging of *N. benthamiana* epidermal cells using laser-scanning confocal microscopy revealed the majority of the putative effectors localized to the nucleus and cytosol. However, fluorescence signal from one candidate effector, PM02_378, accumulated predominantly in the cytosol with weak fluorescence signal detected in the nucleus. We also show that though all candidate effectors expressed detectable protein, none were able to suppress cell death triggered by BAX or INF1 when transiently expressed in *N. benthamiana*, revealing these putative effectors are not likely to function as general cell death suppressors. Importantly, six candidate effectors consistently suppressed cell surface-triggered immune responses including chitin-dependent reactive oxygen species production and MAP kinase activity, revealing these putative effectors contribute to inhibition of immune responses. These results provide valuable insights into the putative functions of candidate effectors from *P. maydis* and will likely stimulate new research aimed at elucidating the molecular mechanisms potentially manipulated by this fungal pathogen.

Funding acknowledgement: United States Department of Agriculture (USDA)

P271

In situ quantification of plant carbon allocation in a Maize-AMF system

(submitted by Kong Wong <kwong@danforthcenter.org>)
Full Author List: Wong, Kong M.[1]; Duncan, Keith E.[1]; Komarov, Sergey[2]; Daniels, Dierdra[3]; Lebow, Clara[1]; Floss, Daniela S.[3]; Tai, Yuan-Chuan[2]; Topp, Christopher N.[1]
[1] Donald Danforth Plant Science Center, St. Louis, MO, 63132 USA
[2] Department of Radiology, Washington University in St. Louis, St. Louis, MO, 63110 USA
[3] Valent BioSciences Corporation, Libertyville, IL, 60048 USA

Plants engage in symbiosis with arbuscular mycorrhizal fungi (AMF) to exchange plant-derived carbon for essential inorganic nutrients, such as nitrogen and phosphorus. In addition, this symbiosis may sequester carbon in root and microbial biomass and improve plant tolerance to abiotic and biotic stresses. The carbon cost of this nutrient exchange likely depends on the AMF strain, and the partitioning of plant-derived carbon to fungal storage organs differs by AMF strain. A better understanding of the fate of carbon within plant-AMF systems will enable the optimization of AMF consortia for carbon sequestration and plant growth. However, quantification of AMF effects on plant carbon allocation is limited, and most methods require destructive sampling. Here, we combine x-ray computed tomography (XCT) and positron emission tomography (PET) to observe and quantify in situ the flow of carbon from leaves to roots to hyphae. Preliminary results in a maize-Rhizophagus irregularis system suggest that R. irregularis induces an increase in carbon allocation to the root system than in uninoculated controls. Comparison of the segmented root systems obtained from XCT images indicates an increase in lateral root growth in inoculated plants. Co-registered XCT and PET images suggest the increase in carbon correlates with increased lateral root development near the inoculation site. Our in situ method for quantifying carbon allocation has deepened our understanding of maize-AMF symbiosis and will enable future studies characterizing how different AMF strains alter carbon partitioning in various land plants.

Funding acknowledgement: Valent BioSciences Corporation, Sumitomo Chemical Corporation

P272

Inoculation with selected strains of *Bacillus megaterium* and *B. subtilis*, present in the first inoculant released in Brazil, increases maize phosphorus acquisition and yield

(submitted by Sylvia Morais de Sousa Tinoco <sylvia.sousa@embrapa.br>)
Full Author List: Oliveira-Paiva, Christiane A[1]; Tinoco, Sylvia M S[1 2]; Gomes, Eliane A[1]; Santos, Flavia C[1]; Lana, Ubiraci G P[1]; Ribeiro, Vitoria P[2]; Bini, Daniel[1]; Pinto Jr, Artur S[3]; Marriel, Ivanildo E[1 2]
[1] Embrapa Maize and Sorghum, Rod MG 424 Km 45, Sete Lagoas, MG, 35701-970, Brazil
[2] Universidade Federal de São João del-Rei, R. Padre João Pímentel, 80 - Dom Bosco, São João del Rei - MG, 36301-158, Brazil
[3] Simbiose-Agro, BR 158 Km 206 - Distrito Industrial CP 820, Cruz Alta – RS, 98005-970, Brazil

Phosphate solubilizing microorganisms are the one of the most sustainable and inexpensive alternatives for enhancing phosphorus (P) availability for plants once they can transform insoluble P into soluble forms by different mechanisms. In this work, we analyzed the effect of the inoculation of two bacterial strains (*Bacillus subtilis*_CNPMS B2084 and *B. megaterium*_CNPMS B119), isolated from P-efficient tropical maize genotypes, on maize growth. The two *Bacillus* strains were co-inoculated under controlled conditions and enhanced total root area. The *Bacillus* strains were inoculated separately and co-inoculatedd in maize in two different localities (Sete Lagoas and Goiânia, Brazil) that represent two crop growing regions, in three seasons in a completely randomized block design with four replicates. *Single inoculation* of maize plants with CNPM B119 and B2084 increased grain yield by 14% and 10%, respectively. The co-inoculation of the two strains in maize increased grain P content by 13% and grain yield by 17%. Additionally, soil P-cycling and P availability were significantly higher after the third year of inoculation. These positive results led to the release in 2019 of the first Brazilian commercial P-solubilizing bacteria inoculant.

Funding acknowledgement: Fapemig (Grant No MPR- 00172-16); Embrapa (Grant No 01.13.05.001.02-0, Simbiose) and CNPq/INCT-Plant-Growth Promoting Microorganisms for Agricultural Sustainability and Environmental Responsibility (Grant No 465133/2014-2)

P273

Insecticidal 3-deoxyanthocyanidin flavonoids from maize and sorghum to fanage fall armyworm

(submitted by Tyler Lesko <tkl5215@psu.edu>)
Full Author List: Lesko, Tyler[1]; Chatterjee, Debamalya[1]; Peiffer, Michelle[2]; Felton, Gary[2]; Chopra, Surinder[1]
[1] Department of Plant Science, The Pennsylvania State University, University Park, PA 16802, USA
[2] Department of Entomology, The Pennsylvania State University, University Park, PA 16802, USA

Flavonoids are a group of polyphenolic secondary metabolites produced by plants, typically as pigments that attract pollinators or as defensive compounds. The phenylpropanoid pathway is responsible for the synthesis of flavonoids. Various sub-branches of this pathway form several classes of flavonoids including flavan-4-ols (phlobaphenes), 3-deoxyanthocyanidins (3-DAs), and flavan-3,4-diols (anthocyanins). The pathway begins with phenylalanine leading to the production of naringenin, where the pathway splits to produce phlobaphenes or anthocyanin depending on the genotype. In maize, a MYB transcription factor *pericarp color1* (*p1*) regulates flavan-4-ols in floral tissue such as kernel pericarp and cob glumes. In sorghum, *yellow seed1* (*y1*), an orthologue of *p1*, regulates the accumulation of flavan-4-ols and 3-DAs in floral as well as vegetative tissues. Our previous study showed that *Y1*-induced 3-DAs in sorghum are deleterious to corn leaf aphids and anthracnose leaf blight. We developed a methodology to extract bioactive 3-DAs from sorghum leaves expressing *yellow seed1*. We tested the efficacy of the extracted sorghum 3-DAs against fall armyworm larvae by supplementing them in an artificial diet and by topically spraying leaves of FAW susceptible maize lines. The results showed high mortality and reduced growth of FAW larvae. Further, we found that the survival of FAW larvae was reduced when fed on transgenic *Y1*-maize as well as flavonoid-overproducing maize breeding lines as compared to wild type near-isogenic lines. Interestingly, the peritrophic membrane that surrounds the food bolus was damaged in the flavonoid-fed larvae, possibly contributing to mortality. Together, our results confirmed the efficacy of sorghum 3-DA extract and endogenous maize flavonoids against the survival of FAW larvae. Our current research is aimed to decipher the mechanisms underlying high mortality and reduced growth of FAW larvae mediated through flavonoids. Our overarching goal is to contribute to ecologically sustainable FAW management strategies.

Funding acknowledgement: United States Department of Agriculture (USDA)

P274

Loss of Allene Oxide Cyclase results in developmental changes and hypersensitivity to fungal and insect pests due to jasmonic acid deficiency

(submitted by Charles Hunter <cthunter3@gmail.com>)
Full Author List: Hunter, Charles T[1]; Li, Q.B.[1]; Christensen, Shawn[1 2]; Gorman, Zachary[1]; Block, Anna K[1]
[1] Chemistry Research Unit, USDA-ARS; Center for Medical, Agricultural and Veterinary Enotomology; Gainesville, FL, 32608
[2] Nutrition, Dietetics & Food Science; Brigham Young University; Provo, UT, 84602

Jasmonic acid (JA) and its derivatives are important regulators of plant defense and development. As part of our efforts to gain insights into JA control of plant defenses we have used CRISPR/Cas9-based gene editing to eliminate key points in JA biosynthesis. Allene oxide cyclases (AOC) convert allene oxide to 12-oxo-phytodienoic acid (12-OPDA), representing the first committed step towards JA biosynthesis. Phylogenetic analysis revealed that two highly similar genes make up the AOC gene family in maize. Gene-edited single mutants indicate that the two maize AOC genes are largely redundant, with mutations in either gene appearing phenotypically wildtype. Expression analysis supports redundancy between the genes, with the two having largely overlapping expression patterns. Double mutants, however, show characteristic phenotypes of JA deficiency, including complete feminization of male florets, resulting in a tasselseed phenotype. Double mutants also exhibit increased susceptibility to biotic challenge. Fall armyworm larva grow at more than double the rate when fed on AOC double mutant leaf tissue. Similarly, leaf spot inoculations of the necrotrophic pathogen *Cochliobolus heterostrophus* (Southern Leaf Blight – SLB) showed that AOC double mutants were highly susceptible to fungal infection. Analysis of herbivory-induced plant volatiles showed reductions in sesquiterpene volatiles. All together, these phenotypes indicate a general reduction in JA-modulated defense responses in AOC-deficient mutants. Interestingly, metabolic examination of mutants revealed dramatically less JA being produced after wounding or herbivory, though not complete loss of 12-OPDA and JA. We speculate that there exist alternative paths for cyclization of allene oxide into 12-OPDA.

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

P275

Low-cost photosynthetic fluorescence phenotypic solutions for GWAS

(submitted by Fangyi Li <fangyihuskers@gmail.com>)
Full Author List: Li, Fangyi[1 2 3]; Grzybowski, Marcin[1 3]; Roston, Rebecca[2 3]; Schnable, James C.[1 3]
[1] Department of Agronomy and Horticulture, University of Nebraska–Lincoln, Lincoln, NE 68588
[2] Department of Biochemistry, University of Nebraska–Lincoln, Lincoln, NE 68588
[3] Center for Plant Science Innovation, University of Nebraska–Lincoln, Lincoln, NE 68588

Plant photosynthetic productivity is a key constraint on plant growth, development, and yield. Photosynthetic activity is highly sensitive to changes in environment including osmotic status, temperature, and light intensity. The rapid changes in photosynthesis combined with the high cost and long measurement times required to measure photosynthetic parameters have limited the study of natural variation in photosynthetic parameters under field conditions. Here we evaluate the feasibility of a low-cost instrument for quantifying photosynthetic fluorescence phenotypes in a large maize diversity panel. Measurements of Fv'/Fm' made with the low-cost instrument and gold standard sensors across a small panel of diverse genotypes under multiple treatments in controlled environments were highly correlated. In the field, a large proportion of total variability of observed Fv'/Fm' was explained by variation in light intensity and temperature throughout the three-day collection in 2020, as well as instrument to instrument variation. In the improved experiments, the initial dataset conducted over three nights in 2021 was augmented with the data from one night collection in 2022. The results show a large proportion of variation in many photosynthetic fluorescence parameters being explained by genetic factors, where the heritability of Fv'/Fm' reached 0.39 in 2021 and 0.41 in 2022. Genome wide association studies conducted with the resulting photosynthetic fluorescence traits identified a number of significant loci distributed across the maize genome.

Funding acknowledgement: NU wheat foundation board

P276

Maize rough endosperm6 (rgh6) is predicted to affect RNA processing in endosperm development

(submitted by Tianxiao Yang <tianxiao.yang@ufl.edu>)
Full Author List: Yang, Tianxiao[1 2]; Suzuki, Masaharu[1 2]; Hannah, L.Curtis[1 2]; Settles, A. Mark[1 2 3]
[1] Plant Molecular and Cellular Biology Program, University of Florida, Gainesville, Florida, USA
[2] Horticultural Sciences Department, University of Florida, Gainesville, Florida, USA
[3] Bioengineering Branch, NASA Ames Research Center, Moffett Field, California, USA

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 isolated the *rgh6* mutant from the UniformMu transposon tagging population. The *rgh6* mutant has reduced kernel weight with defective embryos that fail to germinate, suggesting *rgh6* mutation is a lethal mutation. We mapped the *rgh6* locus within a 60 kbp interval on chromosome 5. The *rgh6* locus has a *Mutator* (*Mu*) transposon insertion within a predicted DEAD-box RNA helicase gene. A second allele of *rgh6* locus was identified from UniformMu population. Both alleles segregate mutant kernels at frequency consistent with recessive mutation. Crosses of the two alleles fail to complement the kernel phenotype, suggesting *rgh6* mutation disrupt endosperm development. The *RGH6* transcripts are not detected in mutant endosperms, and the RGH6 protein is localized to the nucleolus. Further enzymatic activity of the RGH6 protein and transcriptome impact of the *rgh6* mutant are needed to elucidate the roles of RNA processing in endosperm development.

Funding acknowledgement: United States Department of Agriculture (USDA)

P277

Maize transcriptional and microRNA regulation by gibberellic acid

(submitted by Cassandra Eddy <cassie.eddy01@gmail.com>)
Full Author List: Eddy, Cassandra G.[1]; Elder, Jim M.[2]; Best, Norman B.[2]
[1] University of Missouri, Columbia, MO, USA 65201
[2] USDA-ARS, Plant Genetics Research Unit, Columbia, MO, USA 65201

Phytohormones are plant growth regulators produced by plants that regulate important physiological processes. Gibberellic acid (GA) is a phytohormone that plays a role in the regulation of stem elongation, leaf development, and germination. In doing so, GA must coordinate with other hormones and physiological pathways. GA signaling acts through DELLA proteins that can promote or repress expression of target genes through the physical interaction with other transcriptional regulators. miRNAs are a type of non-coding RNA that also play an important role in gene regulation. GA has been previously shown to have a reciprocal relationship with miRNAs, with GA regulating expression of miRNAs or their target genes and miRNAs also targeting components of GA pathways. This project attempted to explore changes in levels of RNA and miRNAs correlated with high levels of exogenous GA applied as a soil treatment. A concentration curve was performed to determine an optimal concentration of 600 micromolar GA_3 solution to highly affect plant growth. GA solution was added to seedlings as a soil drench and tissue from below the first leaf collar was collected for both control and experimental groups after 24 hours of treatment. RNA and miRNAs from each group were extracted and sequenced. Results will provide a better understanding of the impact that hormones, specifically GAs, have on maize transcriptional responses.

Funding acknowledgement: United States Department of Agriculture (USDA)

P278

Molecular characterization of a common rust effector AvrRp1-D recognized by maize immune receptor Rp1-D

(submitted by Saet-Byul Kim <saetbyul.kim@unl.edu>)
Full Author List: Kim, Saet-Byul[1 5]; Kim, Ki-Tae[2]; In, Solhee[3]; Park, Eunsook[3]; Balint-Kurti, Peter[4 5]
[1] University of Nebraska-Lincoln, Lincoln, NE, 68583
[2] Sunchon National University, Suncheon, South Korea 57922
[3] University of Wyoming, Laramie, WY, 82070
[4] Plant Science Research Unit, USDA-ARS, Raleigh, NC, 27695
[5] NC State University, Raleigh, NC, 27695

Common rust, caused by the obligate biotroph *Puccinia sorghi*, is one of the destructive diseases of maize. Alleles of the maize *Rp1* resistance gene confer race-specific resistance to *P. sorghi*. *Rp1-D* is a resistance protein of coil-coil nucleotide-binding leucine-rich repeat (CNL) type that confers resistance to *P. sorghi* race IN2. The *Rp1-D* dependent resistance response includes a rapid localized cell death at the point of pathogen penetration known as a hypersensitive response (HR). We undertook the identification of the fungal protein recognized by Rp1-D that triggers Rp1-D dependent HR, which we have termed AvrRp1-D. Fungal haustoria are the major site of pathogen/host cell-to-cell contact during pathogenesis and are the major source of pathogen proteins introduced into host cells. We used a novel technique using biotinylated concanavalin A, which binds to fungal cell walls, to isolate haustoria from susceptible maize leaves infected with *P. sorghi* IN2. Haustorial RNA was isolated, and RNA-seq was performed for de novo transcriptome assembly. We identified 250 *AvrRp1-D* candidates using bioinformatic approaches. Then, we performed a high-throughput screening assay in *Nicotiana benthamiana* to identify genes that induce Rp1-D-dependent cell death. A candidate AvrRp1-D gene was identified, and we confirmed its ability to induce *Rp1-D* dependent cell death in maize protoplasts. To better understand the interaction between Rp1-D and AvrRp1-D, *AvrRp1-D* was co-expressed with six other *Rp1* alleles that do not confer resistance to *P. sorghi* race IN2. As expected, none of these alleles induced strong HR when co-expressed with *AvrRp1-D*. Yeast two-hybrid assays suggested that the C-terminal part of the leucine-rich repeat of Rp1-D directly interacted with AvrRp1-D and was responsible for specificity amongst different *Rp1* alleles. Thus, our study has identified a novel pathogen-derived protein that triggers *Rp1-D*-dependent cell death through direct interaction.

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

P279

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

(submitted by Tyler Ferris <tyler.ferris@huskers.unl.edu>)
Full Author List: Ferris, Tyler W[1]; Hurst, Preston J[1]; Thompson, Rachel[1]; Li, Axia[1]; Clemente, Thomas E[1]; Holding, David R[1]
[1] University of Nebraska-Lincoln, 1901 Vine St, Lincoln, Nebraska, 68588

Grain sorghum (Sorghum bicolor (L.) Monech) is a crop of tremendous significance. Grain sorghum is cultivated for consumption by humans and livestock alike and is valued for its robust 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 the 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. 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 resulted in a reduction in kafirin expression in endosperm cells and elicited a proteome re-balancing effect 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 in order to improve the dough-making potential of sorghum grain. The individual goals of this research are as follows. 1) To test for protein quality, digestibility, amino acid content, and starch composition, and 2) to perform rheological analysis on dough. Currently, the marker to detect k1C-family editing has been developed and used to select F2 plants with the introgressed waxya mutation. F4 seeds will be used in protein digestibility testing, amino acid profiling, dough rheology analysis, in vitro protein digestibility and starch composition testing . The results of these tests will provide insight into the nutritional and biophysical properties of sorghum grain produced from this novel combination of alleles.

Funding acknowledgement: United States Department of Agriculture (USDA)

P280

Proteomic and metabolomic landscape of association between maize root and arbuscular mycorrhizal fungi

(submitted by Rohit Kumar <mohank@clemson.edu>)
Full Author List: Kumar, Rohit[1]; Tharayil, Nishanth[1]
[1] Department of Plant and Environmental Sciences, Clemson University, Clemson, SC, USA 29634

Phosphorus is the second most important macronutrient and plays a vital role in the plant growth cycle. The phosphate supplied to plants in the form of fertilizers is mainly obtained from natural phosphate reserves which are declining rapidly. Therefore, to maintain sustainable crop production, we must utilize the available phosphate resources efficiently. One of the promising approaches to meet this target is to increase the phosphate use efficiency, which is the utilization of available phosphate in the soil by the plants. Arbuscular mycorrhizal fungi (AMF) and plants form a symbiotic relationship that promotes phosphate utilization by crop plants. However, the regulatory mechanisms through which AMF promotes phosphate utilization in plants are largely unknown. In this study, we used time course proteomics and metabolomics to analyze the maize roots grown with and without AMF inoculation. We identified multiple differentially expressed proteins and metabolites involved in mediating the interaction between AMF and roots. Validation of the metabolites and proteins is underway using different tools and databases. Our study will help in bridging the gap in understanding the mechanisms and signaling underlying root mycorrhizae interaction for efficient phosphate utilization.

Funding acknowledgement: United States Department of Agriculture (USDA)

P281 @mae_mercadow

RUBISCO activase3 (*rca3*) and its potential role in heat stress response in maize

(submitted by Mae Antonette Mercado <mercado6@illinois.edu>)
Full Author List: Mercado, Mae Antonette G.[1]; Bhatnagar, Nikita[2]; Sands, Mia[2]; Burgess, Steven[2]; Ort, Donald[2]; Studer, Anthony J.[1]
[1] Department Crop Sciences, University of Illinois Urbana-Champaign, USA, 61801
[2] Carl R. Woese Institute for Genomic Biology, University of Illinois at Urbana-Champaign, USA, 61801

Rubisco activase (RCA) is a protein that activates Ribulose-1,5-bisphosphate carboxylase-oxygenase (RUBISCO) by removing sugar phosphates and has been hypothesized to be involved in heat stress response in plants. However, the role of RCA in heat stress has not been experimentally determined in C_4 grasses like maize. Some plant species have a single *Rca* gene that undergoes alternative splicing, but in maize, two *Rca* genes encode the α and β isoforms. The β isoform is encoded by *rca1* (Zm00001eb164390), while the α isoform is encoded by *rca3* (Zm00001eb164380) and is up regulated during heat stress. Here we present the characterization of *rca3* using both *Dissociation* (*Ds*) and *Mutator* (*Mu) transposable element insertion alleles.* Sanger Sequencing identified the site of the *Ds* insertion (*rca3-m2::Ds*) in the second exon, and the *UniformMu* insertion (mu1022546) in the third exon of *Zmrca3*. Characterization of the mutant lines indicate that the insertions disrupted *rca3* expression at 42°C. Gas exchange measurements at 42°C were used to identify variability between the response of the wildtype and mutant lines to heat stress. Leaf-level gas exchange measurements taken at three different time points (two hours after dawn, four hours after heat treatment and four hours after recovery) showed that the *rca3-m2::Ds* mutant plants have significantly lower photosynthetic rates after being exposed to high temperature for four hours. We also observed that the photosynthetic rate after recovery only reached 80% of its initial value. The Uniform*Mu* allele had a less severe phenotype than the *Ds* allele, which we attribute to the position of the insertion, which creates a knockdown rather than a knockout of *Rca3* expression. Our understanding of the role of RCA in heat stress will guide the improvement of crops that can be more resilient to future temperature fluctuations.

Gene / Gene Models described: *RUBISCO activase3 (rca3)*; Zm00001eb164380
Funding acknowledgement: Department of Energy (DOE), CABBI, University of Illinois Urbana-Champaign, Illinois Corn Growers Association

P282

Roles of REL2 mediated transcriptional co-repression in maize immunity

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

Protein acetylation is a major post-translational modification that modulates many cellular processes, including plant immunity and stress responses. *Cochlibolus carbonum* (Northern Corn Leaf Spot) produces the effector HC-Toxin, a lysine deacetylase inhibitor required for pathogen virulence. RAMOSA1 ENHANCER LOCUS2 (REL2) is a transcriptional corepressor homologous to TOPLESS (TPL) in *Arabidopsis*. TPL family members are required for a range of biological processes, including development and immunity, and are critical components of hormone responses, including auxin and jasmonate signaling pathways. We identified a lysine acetylation site on REL2 using global acetylome profiling of maize treated with HC-Toxin or *C. carbonum*. Furthermore, we found that *rel2* loss of function mutant plants are susceptible to infection, demonstrating that REL2 is directly related to plant immunity. This work aims to elucidate how hyperacetylation impacts the biological activity of REL2 and REL2's roles in plant-pathogen interactions. Specifically, I will determine REL2-associated gene expression and elucidate how REL2 acetylation state impacts maize immunity. These objectives will be completed via proteomics, high-throughput sequencing, biochemical, and genetic experiments to further 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*; GRMZM2G042992, Zm00001d024523
Funding acknowledgement: National Science Foundation (NSF)

P283
Seed expressed scorable markers facilitating seed sorting to accelerate plant biology research

(submitted by Nathalie Walter <nwalter8888@gmail.com>)
Full Author List: Collier, Ray[1]; Li, Guifen[1]; Walter, Nathalie[1]; Peickert, Aimee[1]; Ball, Hannah[1]; Mahoy, Jill[1]; Harnish, Robert[1]; Kaeppler, Shawn[1]
[1] University of Wisconsin Crop Innovation Center Middleton WI USA 53562

Recent technologies are allowing researchers to make process improvements to the plant transformation pipeline that has historically limited advancement of plant biology research. Improved plant transformation might subsequently shift the bottleneck from event generation to analysis of the transgenic plants. Tools which allow researchers to quickly sort transgenic from null segregant T_1 siblings support plant analysis and experimental logistics, thus partially mitigating the potential negative impacts of a bottleneck shift. Rapid identification of null segregants produced in CRISPR/Cas9 experiments is especially useful since edited plants in which the gene editing machinery has segregated away from the edit support experiment implementation and regulatory compliance. Previous studies identified and characterized the maize a-zein promoter, and the soybean GmScream6 promoter, both of which have been shown to limit gene expression to seeds. The Wisconsin Crop Innovation Center (WCIC) Molecular Technologies Department has leveraged this information by coupling these seed specific promoters to either the fluorescent protein tandem Tomato, or the recently reported RUBY polycistron which directs the synthesis of the chromogenic compound betanin. The WCIC Production Transformation Team generated transgenic T_0 maize and soybean plants using constructs containing the seed specific promoter driven scorable markers. T_1 seeds produced by the T_0 plants were examined for scorable marker presence. We found that expression of tdTomato and RUBY was reliably confined to maize kernels or soybean seeds, and that overall health and vigor of the T_0 plants was not compromised. Interestingly, we found in separate experiments that when RUBY was constitutively expressed in maize the plants were obviously negatively impacted, indicating that tissue specific expression of RUBY might be necessary. The proven seed marker transcriptional units will now be deployed in DIRECTCLONE binary plasmids which will soon be deposited in the Addgene repository for public access and use

Funding acknowledgement: National Institutes of Health (NIH)

P284 @KhanguraRajdeep
Semi-dominant maize mutant *Bella fleck1* provides resistance to multiple fungal diseases

(submitted by Rajdeep Khangura <rkhangur@purdue.edu>)
Full Author List: Khangura, Rajdeep S[1 2]; Benke, Ryan L[1 2]; Olomukoro, Enameguono E[1 2]; Meline-Gomez, Paula[3]; Aime, Mary C[3]; Johal, Gurmukh S[3]; Dilkes, Brian P[1 2]
[1] Department of Biochemistry; Purdue University; West Lafayette, IN, USA 47907
[2] Center for Plant Biology; Purdue University; West Lafayette, IN, USA 47907
[3] Department of Botany and Plant Pathology; Purdue University; West Lafayette, IN, USA 47907

Lesion-forming (*les*) mutants of maize produce spontaneous lesions or speckles on the leaf blade and sheath in the absence of pathogens. Over 30 *les* mutant complementation groups have been recovered but seven have been molecularly identified. These genes disrupt porphyrin and chlorophyll metabolism and encode auto-active NBS-LRR proteins and a protein of unknown function. In all characterized *les* mutants, lesion initiation and severity increase as the leaves age. We have identified a novel semi-dominant lesion mutant, *Bella fleck1* (*Bfl1*), that forms white flecks in newly emerged leaves that do not get progressively more severe as the leaves age. Heterozygous *Bfl1*/+ mutants are stunted, exhibit suppressed nodal root growth, and show high lodging incidence. The *Bfl1*/+ plants are resistant to common rust, physoderma brown spot, and common smut but susceptible to southern rust, northern corn leaf spot, northern corn leaf blight, and grey leaf spot. Consistent with a role in biotic resistance, we found that the global gene expression pattern and metabolite accumulation in *Bfl1*/+ was similar to *Rp1-D21*/+, a maize lesion mutant encoded by a hyperactive NBS-LRR protein. The similarities include the accumulation of defense-responsive metabolites and transcripts, demonstrating that *Bfl1* functions in plant immunity. We mapped *Bfl1* to the long arm of chromosome 2. Association mapping of modifiers of *Bfl1*using F1 hybrids between a maize association panel and *Bfl1* identified a cis-acting modifier allele in the region encoding *Bfl1*. The molecular identity of the gene underlying *Bfl1* and its natural modifier is currently being investigated.

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

P285 @NadiaNazihM

Small-kernel ears result when *Sorbitol dehydrogenase1 (Sdh1)* is dysfunctional

(submitted by Nadia Mourad Silva <nmourad@ufl.edu>)
Full Author List: Mourad Silva, Nadia N.[1]; de Sousa Tinoco, Sylvia M.[2]; Sanclemente, Maria A.[3]; Gray, Katherine E.[1]; Joe, Sarah E.[1]; Saunders, Jonathan W.[1]; Guan, Jiahn-Chou[1]; Rolletschek, Hardy[4]; Borisjuk, Ljudmilla[4]; Koch, Karen E.[1]
[1] University of Florida, Department of Horticultural Sciences, Fifield Hall 2550 Hull Rd., Gainesville, FL, 32611 USA
[2] Embrapa Maize and Sorghum, Rod MG 424 Km 45, Sete Lagoas, MG, 35701-970 Brazil
[3] Utrecht University, Heidelberglaan 8,3584 CS Utrecht, Netherlands
[4] Institute of Plant Genetics and Crop Plant Research (IPK), Corrensstr. 3, D-06466 Seeland-Gatersleben, Germany

Maize kernels rapidly synthesize and metabolize sorbitol via sorbitol dehydrogenase (SDH, EC 1.1.1.14). Maize SDH catalyzes the reversible interconversion of fructose + NADH ↔ sorbitol + NAD^+. The reaction is suggested to favor sorbitol and NAD^+ synthesis in the high-sugar, low-oxygen interior of the endosperm and possibly the reverse in other locales. However, the physiological roles of sorbitol synthesis and the fate of sorbitol in the maize kernel remain elusive. A single copy *Sdh1* gene encodes maize SDH expressed almost exclusively in the endosperm during grain fill. Preliminary analysis of a new, *Ac/Ds*-induced *sdh1* mutation indicates that it confers a small-kernel phenotype similar to that observed for a *Mu*-induced *sdh1* allele, suggesting a possible role in kernel development. We have also observed enhanced seed set in the *sdh1-Ds* mutant during multiple growing seasons but are yet to define possible mechanisms for this aspect of the *sdh1* phenotype. For physiological effects on kernel size, we hypothesize that maize SDH can aid regeneration of NAD^+ in the low-O_2 endosperm, thus aiding glycolytic flux and helping sustain kernel growth. Characterization and biochemical analysis of *sdh1* mutants are ongoing, with preliminary results showing a ~10x decrease in sorbitol and an increase in fructose and sucrose in the mutant. The physiological and genetic effects of *Sdh1* on kernel development are being further evaluated in over-expression transgenic lines and in sweet corn, where soluble sugar levels are high. Outcomes will determine the suitability of *Sdh1* and its regulators as possible targets for genetic manipulation or metabolic engineering to alter quality and/or quantity of maize kernels.

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

P286

Surprising non-linear phenotypic impacts of variation in chlorophyll affected by natural and induced variation the Mg2+ Chelatase subunit I

(submitted by Brian Dilkes <bdilkes@purdue.edu>) (**Presenter: Rajdeep Khangura**, *Postdoc*)
Full Author List: Dilkes, Brian[1]; Kaur, Amanpreet[1]; Khangura, Rajdeep[1]
[1] Department of Biochemsty and Center for Plant Biology, Purdue University, West Lafayette, IN USA 47907

The semi-dominant mutant allele *Oy1-N1989* encodes a dominant-negative subunit I of Mg-Chelatase that catalyzes the first committed step of chlorophyll biosynthesis. Analyses of mutant F1 hybrids from crosses of *Oy1-N1989* to diverse maize lines identified a natural variant at *oil yellow1* as major modifier of chlorophyll content. Chlorophyll reduction increased the time to reproductive maturity, decreased stalk width, and had complex effects on plant height. We explored these effects by genome wide association studies of F1 hybrids crosses of *Oy1-N1989* to a maize diversity panel. The delay in reproductive maturity in *Oy1-N1989/+* mutants neither altered the vegetative-to-adult phase change nor total leaf number but did slow the rate of organ emergence from the whorl. In addition to the allele at *oy1*, analysis of tetrapyrrole, heme, and chlorophyll biosynthesis pathway genes identified multiple alleles that modify *Oy1-N1989/+* mutant traits. Consistent with organ emergence, rather than a developmental delay, affecting the reproductive delay of *Oy1-N1989/+*mutants, known flowering time regulators did not affect variation in the reproductive delay affected by chlorophyll loss. Plant height has repeatedly shown a complex relationship with chlorophyll. The *Oy1-N1989/+*mutant F1 plants with a modest reduction in chlorophyll were taller than their wildtype siblings, but F1 plants with dramatically decreased chlorophyll were shorter than their wildtype siblings. Synergistic interactions between *elm1*, defective in bilin production, and *Oy1-N1989/+* demonstrate that perturbations in the biosynthesis of the phytochrome chromophore increased plant height in wild-type plants but dramatically reduced plant height and chlorophyll contents of *Oy1-N1989/+* mutants. The mechanism by which the interaction between chlorophyll and bilin affects height and chlorophyll remains to be explored.

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

P287

Targeting changes in strigolactone biosynthesis to confer witchweed resistance without yield penalty in maize

(submitted by Jiahn-Chou Guan <guanjc@ufl.edu>)
Full Author List: Guan, Jiahn-Chou[1]; Gray, Katherine E.[1]; Li, Changsheng[2]; Floková, Kristýna[2]; Dong, Lemeng[2]; Bouwmeester, Harro J.[2]; Koch, Karen E.[1]
[1] Horticultural Sciences Department, University of Florida, Gainesville, Florida, USA 32611
[2] Plant Hormone Biology Group, Swammerdam Institute for Life Sciences, University of Amsterdam, Science Park 904, 1098 XH, Amsterdam, The Netherlands

Maize is a major staple crop in Africa, where its yield and the livelihood of millions are compromised by the parasitic witchweed, *Striga*. Germination of *Striga* seed is induced by strigolactones exuded from maize roots into the rhizosphere. Recently, the entire biosynthetic pathway responsible for producing a full spectrum of maize strigolactones has been decoded (Li *et al*., 2023). Here, we present phenotypic analyses of four mutants in in this SL biosynthetic pathway: Dysfunction of a cytochrome P450 (*ZmMAX1b*) significantly decreases levels of zealactone, a major SL stimulant of *Striga* seed germination. However, double mutants of the other two cytochrome P450s (*ZmMAX1a* and *ZmMAX1c*), produce wild-type levels of zealactone. This genetic evidence supports previous biochemical research demonstrating that *ZmMAX1b* encodes the major enzyme converting carlactone to carlactonic acid. Importantly, the *zmmax1b* mutants induced significantly less *Striga* germination and emergence without discernable disruption to shoot branching or yield. *Striga* resistance was also evident in an NP2222 inbred with a dysfunctional gene for carlactonic acid methyltransferase (*ZmCLAMT1*) (Li *et al*., 2023). Further support for this association was achieved by our recent characterization of a transposon insertional mutant (*zmclamt1*) in this same gene. Results were central to the work by Li *et al.* (above) demonstrating that a change in activity of specific SL biosynthetic enzymes can alter the SL composition and confer *Striga* resistance. The wild-type phenotype of these mutants sheds new light on potentials for witchweed control by highlighting breeding targets. Li, C., Dong, L., Durairj, J., Guan, J. C… *et al*., (2023) Maize resistance to witchweed through changes in strigolactone biosynthesis. Science 379: 94-99.

Gene / Gene Models described: *ZmMAX1a, ZmMAX1b, ZmMAX1c, ZmCLAMT1*; Zm00001d046207, Zm00001d039697, Zm00001d053569, Zm00001d039695

Funding acknowledgement: National Science Foundation (NSF)

P288

The Wisconsin Crop Innovation Center: a public resource for maize transformation and editing service, research and discovery

(submitted by Ray Collier <rcollier2@wisc.edu>)
Full Author List: Walter, Nathalie[1]; Mahoy, Jill[1]; Hahn, Emily[1]; Thompson, Mark[1]; Collier, Ray[1]; Carlson, Alvar[1]; Kaeppler, Heidi[1]; Kaeppler, Shawn[1]
[1] University of Wisconsin Crop Innovation Center Middleton WI USA 53562

The Wisconsin Crop Innovation Center (WCIC), part of the University of Wisconsin – Madison, is a ~100,000 ft^2 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 accelerate the time from transformation to T_1 seeds and will be effective across many maize inbreds. CRISPR/Cas9-based gene editing is one of the outcomes available currently in LH244. RNA guide discovery has been empowered by public deposit of the LH244 genome sequence by Bayer Crop Science. The sequence is now available through the CRISPOR web-based guide RNA search tool upon request of WCIC. Typically, LH244 transformation projects start with binary plasmid vector design and assembly in the WCIC Molecular Technologies Department (MTD). In the near future, DIRECTCLONE plasmids, which enable simplified integration of user genes/guide RNA, will be deposited in the Addgene plasmid repository for public access and use. The MTD uses synthesis and Golden Gate cloning to build binary vectors which are subsequently used to produce Agrobacterium stocks for the Production Transformation Team (PTT). Transgenic plants generated by the PTT are delivered to the Plant Analysis Team for endpoint PCR confirmation of presence of the plant selection marker gene and/or transgene copy number enumeration via droplet digital PCR (ddPCR). Verified transgenic events/plants are raised to maturity by the Greenhouse Team, who work with the client on completion of USDA-APHIS permits allowing for shipment of the transgenic/edited seeds.

Funding acknowledgement: National Science Foundation (NSF)

P289

The potential of the ABA-Hydroxylase gene family for fine-tuning of water use efficiency and drought-related traits in maize

(submitted by Larissa Barl <larissa.barl@tum.de>)
Full Author List: Barl, Larissa[1]; Blankenagel, Sonja[1]; Avramova, Viktoriya[1]; Schön, Chris-Carolin[1]
[1] Plant Breeding, TUM School of Life Sciences, Technical University of Munich, 85354 Freising, Germany

High yielding crop varieties with higher water use efficiency (WUE) and general drought tolerance are needed to increase agricultural sustainability. It has been shown, that WUE, plant growth and germination rates under optimal and drought conditions are affected by altered levels of abscisic acid (ABA). ABA-8'-hydroxylases (ABH) are key enzymes involved in ABA catabolism, therefore significantly influencing ABA levels. In maize (*Zea mays* L.), there are five *ZmAbh* genes, that code for five ABH homologs. The *ZmAbh* genes are expressed in various tissues, including leaves, roots and seeds. Little is known about the specific functions of the respective members of the gene family in maize. To investigate the effects of all the *ZmAbh* genes on drought-related traits and to understand their potentially synergetic or complementary effects, we generated various CRISPR/Cas9 mediated null mutants. We have shown that *ZmAbh4* is predominantly expressed in maize leaves and two *abh4* mutants showed improved intrinsic WUE (iWUE), without apparent penalties in plant growth. First results indicate, that *ZmAbh1* and *ZmAbh2* also play an important role in regulating the plant water balance in leaves. To localize their contribution, we developed an ABA-immunostaining for quantifying ABA on a cellular level in stomata and other structures. This will provide the means to observe the response of different *abh* mutants under optimal and under drought stress conditions.

Funding acknowledgement: German Research Foundation (DFG), Collaborative Research Center 924 (SFB924)

P290

Time-course metabolome variation in genetically diverse inbred lines identify pathways underlying staygreen trait in maize

(submitted by Manwinder Singh Brar <mbrar@clemson.edu>)
Full Author List: Brar, Manwinder S.[1]; Kumar, Rohit[2]; Tharayil, Nishanth[2]; McMahan, Christopher[3]; Sekhon, Rajandeep[1]
[1] Department of Genetics and Biochemistry, Clemson University, Clemson, SC 29634, USA
[2] Department of Plant and Environmental Sciences, Clemson University, Clemson, SC 29634, USA
[3] Department of Mathematical and Statistical Sciences, Clemson University, Clemson, SC 29634, USA

Staygreen is an important trait that improves plant productivity by prolonging the period of photosynthetic assimilation. The physiological and metabolic components that accurately discern phenotypic variation for staygreen in maize remain poorly defined, thus making elucidation of the genetic architecture of this trait difficult. We performed an in-depth analysis of physiological and metabolomic variation captured in a set of genetically diverse staygreen and non-staygreen inbred lines. We identified important intermediate traits and streamlined a phenotyping approach that will help standardize future studies on staygreen in maize and related grasses. Time-course analysis of leaf metabolome during senescence revealed substantial variation for primary and secondary metabolites. Remarkably, global patterns of metabolome were strongly associated with the observed phenotypic differences for staygreen, thus indicating a crucial role of the metabolic processes in senescence. Through in-depth analysis of flux in the secondary metabolism pathway, we identified a candidate gene, anthocyaninless1, that specifies staygreen by facilitating higher anthocyanins accumulation to alleviate oxidative stress during senescence. Characterization of a mutation in the Arabidopsis ortholog of maize gene showed loss of anthocyanins accumulation and associated early senescence. The carbon to nitrogen (C:N) ratio increased early in non-staygreen inbred lines compared to staygreen lines, thus indicating a key role of primary metabolism and the C:N ratio. The identity of metabolites and metabolic pathways underlying staygreen will be discussed. Our findings will identify novel components of biological organization that regulate staygreen in maize.

Funding acknowledgement: National Science Foundation (NSF)

P291

Uncovering the link between chlorophyll synthesis and vitamin E in maize grain

(submitted by Samuel Herr <skh77@cornell.edu>)
Full Author List: Herr, Sam[1]; Li, Xiaowei[1]; Wu, Di[1]; Hunter, Charles T.[2]; Magallanes-Lundback, Maria[3]; Wood, Joshua C.[4]; Kaczmar, Nicholas[1]; Diepenbrock, Christine H.[5]; Buell, C. Robin[4]; DellaPenna, Dean[3]; Gore, Michael A.[1]
[1] Plant Breeding and Genetics Section, School of Integrative Plant Science, Cornell University, Ithaca, NY 14853, USA
[2] USDA-ARS Chemistry Research Unit, Gainesville, FL 32608, USA
[3] Department of Biochemistry and Molecular Biology, Michigan State University, East Lansing, MI 48824, USA
[4] Department of Crop & Soil Sciences, Institute of Plant Breeding, Genetics, & Genomics, University of Georgia, Athens, GA 30602, USA
[5] Department of Plant Sciences, University of California, Davis, CA 95616, USA

Tocopherol is a class of tocochromanols that exhibit vitamin E activity, which is essential for human health and plant fitness. Recently, two protochlorophyllide reductases (por1 and por2) involved in chlorophyll synthesis were identified as large-effect loci controlling the natural variation of total tocopherols in maize grain across the U.S. nested association mapping (NAM) panel and the Ames panel. While the majority of the tocopherol biosynthesis pathway has now been elucidated, the mechanism of phytol synthesis, a key precursor of tocopherols, is still unknown in maize grain, a non-photosynthetic tissue. Herein, we provide evidence to support both the hypothesis that the phytol required for tocopherol synthesis is dependent on a chlorophyll-based cycle in the embryo of maize grain and the major role that por genes play in this cycle. We performed two experiments, an environmental knockout and a CRISPR/Cas9 knockout, to observe the metabolomic and transcriptomic responses to inhibiting the function of the por genes. For the environmental knockout experiment, we studied how excess light or light deprivation affected tocopherol abundance compared to control in seven genotypes containing contrasting por effects. We found that light-deprived kernels and embryos had significantly lower chlorophyll a and tocopherol levels compared to the high-light treatment and control. The transcriptomic responses were largely dependent on the genotype. For the CRISPR/Cas9 knockout experiment, the por1/por2 double mutant had reduced tocopherol and chlorophyll a levels in the embryos compared to the wild type. These findings contribute strong evidence that tocopherol synthesis relies on a chlorophyll-based cycle in the embryo of maize grain, suggesting that por genes are important for the biofortification of vitamin E levels in the grain of maize and potentially other cereals.

Gene / Gene Models described: *por1*,*por2*; Zm00001eb357390,Zm00001eb429750
Funding acknowledgement: National Science Foundation (NSF), United States Department of Agriculture (USDA), the National Institute of Food and Agriculture, United States Agency for International Development (USAID), Cornell University

P292

Understanding the molecular and evolutionary mechanisms of epicuticular wax in sorghum

(submitted by Ran Tian <rtian@ttu.edu>)
Full Author List: Tian, Ran[1]; Jiao, Yinping[1]
[1] Department of Plant and Soil Science, Institute of Genomics for Crop Abiotic Stress Tolerance (IGCAST), Texas Tech University, Lubbock, TX, 79409 USA

Sorghum is known as the “camel” of cereal crops for its ability to grow in arid soils and withstand prolonged droughts, and also more heat-tolerant than other major crops including maize. Cuticular wax is the first defense line of sorghum against environmental threats including drought, heat stress, UV light, etc. Interestingly, sorghum produces a much thicker layer of cuticular wax on the leaves and leaves sheath than other cereal crops such as maize and rice per leaf area and weight bases. To have a comprehensive understanding of the molecular mechanism of cuticular wax in sorghum, we are performing a genome-wide dissection by combining large-scale genomics, biochemistry and plant physiology approaches. From our large mutant population, over 100 sorghum bloomless mutants with reduced wax load were identified. So far, a total of 23 causal genes including nine novel genes for bloomless (no epicuticular wax) or sparse bloomless (reduced epicuticular wax) phenotypes have been identified by the based bulk segregation analysis (BSA). The candidate genes have been verified to be the causal gene for the reduced amount of wax by complementation or co-segregation test. The causal genes will be linked together as a network according to the transcriptome data (RNA-seq) and the change of the wax composition (GC-MS/LC-MS) of each mutant. The comparison of the wax biosynthesis pathway in the grass family will indicate the evolution of the epicuticular wax and explain how sorghum could produce more wax than maize. More data will be presented at the conference.

Funding acknowledgement: The State of Texas Governor's University Research Initiative (GURI)

P293

Understanding the genetic architecture controlling seed amino acid composition in arabidopsis

(submitted by Ha Duong <hndvw5@umsystem.edu>)
Full Author List: Duong, Ha N.[1]; Ansaf, Huda[1]; Shrestha, Vivek[1]; Chan, Yen On[1]; Yobi, Abou[1]; Slaten, Marianne L.[1]; Angelovici, Ruthie[1]
[1] Division of Biological Sciences, Interdisciplinary Plant Group, Christopher S, Bond Life Sciences Center, University of Missouri, Columbia, MO 65211, USA

Seeds of staple crops are an important nutritional source, but most are insufficient in several essential amino acids (EAAs) to meet humans' and animals' diets requirements. One of the approaches to improve the seed EAAs levels is to alter the protein-bound amino acids (PBAAs) levels mainly stored in seed storage proteins (SSPs), which are highly abundant but poor in nutritional value. Due to the proteomic rebalancing phenomenon, these approaches have met limited success. Additionally, despite the robustness in a given genotype, PBAA composition natural variation across natural and artificial populations was observed. These suggested that PBAA levels regulations rely on a complex genetic network and can be potentially manipulated. In this study, we harnessed natural variation to an understanding of the genetic regulation of PBAA composition and its potential interplay with FAA in the *Arabidopsis* seed model system. The genetic and phenotypic diversities of the *Arabidopsis* 1001 population were harnessed to complete GWAS on 274 derived biochemical traits. Our data do not reveal a considerable overlap between the FAA and PBAA genetic architectures indicating separate metabolic and genetic regulations. Nevertheless, one QTL was strongly associated with several FAA and PBAA-related traits suggesting a key role in amino acid metabolism. The ongoing effort to understand the role of the three genes associated with this key QTL is elaborated below.

Funding acknowledgement: National Science Foundation (NSF)

P294

Unravelling the relationship between stomatal properties and *Cercospora zeae-maydis* infection in maize

(submitted by Betina Debastiani Benato <betina.benato@tum.de>)
Full Author List: Debastiani Benato, Betina[1]; Avramova, Viktoriya[1]; Schön, Chris-Carolin[1]
[1] Technical University of Munich, School of Life Sciences, Chair of Plant Breeding, Liesel-Beckmann-Straße 2, Freising, Bavaria, Germany, 85354

Maize is the most important crop plant with close to a billion Mt grain produced per year. The gray leaf spot (GLS), caused by *Cercospora zeae-maydis,* is a widespread and damaging foliar maize disease. In Africa and South America, the reported yield losses reached up to 60% and with the changing climate the disease is likely to spread to other growing areas. To establish infection, *C. zeae-maydis* orients hyphal growth toward host stomata and initiates entry into mesophyll tissues. Even though stomata are essential to establish infection, the role played by different stomatal properties as conductance, density and size are unknown. We generated near isogenic lines (NILs) that differ in the above-mentioned stomatal properties. Investigating stomata pattern and disease progression among our NILs and known susceptible and resistant genotypes will allow us to further comprehend the relevance of stomatal properties in the infection process and their role in disease resistance. We established a protocol that allows to quantitatively measure fungus DNA on infected leaves and score GLS. Preliminary results show that levels of infection vary among the studied genotypes depending on their stomatal properties.

Funding acknowledgement: Bavarian State Ministry of the Environment and Consumer Protection within the project group BayKlimaFit 2

P295 @AmanArora_7

Using gene expression patterns as signatures of a biological response reveals the rheostat-like nature of brassinosteroid signaling

(submitted by Amanpreet Kaur <kaur60@purdue.edu>)
Full Author List: Kaur, Amanpreet[1 2]; Best, Norman[3]; Dilkes, Brian[1 2]
[1] Department of Biochemistry, Purdue University, West Lafayette, IN
[2] Center for Plant Biology, Purdue University, West Lafayette, IN
[3] USDA-ARS, Columbia, MO

Plants respond to external or internal stimuli by reprogramming gene expression to elicit a physiological or biochemical response. Transcript profiling captures these changes as gene expression changes affected by a specific phenotype or treatment. The resulting data can be analyzed to uncover signaling mechanisms underlying the phenotype or treatment. However, identifying differentially expressed genes does not provide a comprehensive view of pathway-level effects, quantify the response, or detail the phenotypic impacts. We propose that analyzing coordinated transcript abundance changes in a group of genes can provide this insight. Incorporating prior knowledge of known effects on gene sets provides biological context to gene expression consequences and facilitates the identification of links between gene expression and phenotype. Here, we demonstrate a gene set testing approach to assess the status of brassinosteroid (BR) hormone signaling. The transcriptional consequences of loss of BRs were analyzed by RNA-sequencing of the *brd1* mutant. Genes responsive to BR treatment were obtained from publicly available datasets of BR-treated maize seedlings. The expression of BR induced genes was lower in *brd1* while BR suppressed genes were increased in *brd1,* consistent with loss of BR response in the mutant. To complement this gene set testing, we created a summary statistic called an "index" that aggregates the response of maize genes to BR excess and thus serves as a reporter of BR signaling status. This index calculation turned a set of differentially expressed genes responding to BR excess into a single reporter of BR signaling status, that was utilized to assess BR signaling in our RNA seq samples. BR indices revealed opposing effects on gene expression in BR-treated and BR-deficient samples and index values could assign function to a variety of BR signaling and biosynthetic mutants, demonstrating a rheostat-like nature of BR signaling in maize.

Funding acknowledgement: Department of Energy (DOE)

P296

Genetic markers associated with leaf angle and tassel branch number in maize

(submitted by Mirai Maeda Inaoka <minaoka2@illinois.edu>)
Full Author List: Inaoka, Mirai Maeda[1]; Lipka, Alex E.[1]
[1] Department of Crop Sciences, University of Illinois at Urbana-Champaign, Champaign, IL 61820

Leaf angle (LA) and tassel branch number (TBN) are important traits for improving grain yield in maize (Zea mays L.). To assist plant biologists with understanding which genes control LA and TBN and then incorporate this knowledge into breeding decisions, it is crucial to pinpoint which regions of the maize genome are most likely to contain loci associated with these two traits. We are currently performing a genome-wide association study (GWAS) to identify a subset of markers across the maize genome that exhibit statistically significant associations with LA and TBN. Thus far, the observed results indicate that there are statistically significant marker-trait associations for LA and TBN, suggesting the presences of several nearby large-effect genes. These results will aid in plant breeders' efforts in the future when they are breeding for maize plants that contain the appropriate ratio of LA to TBN to ensure high grain yield.

Author List

Participant List

Participant	Organization
Aaron Avalos Calleros, Jesus	Kansas State University
Aboobucker, Siddique	Iowa State University
Abraham-Juarez, Jazmin	Langebio Cinvestav
Acharya, Bimala	Iowa State University
Adolfo Silva-Villatoro, Pablo	Cinvestav – UGA Langebio
Albertsen, Marc	Albertsen Crop Genetics for Humanity, LLC
Ali, Waqar	University of Nebraska Lincoln
Ali, Sulaiman	Ministry of Agriculture and Water Resources-KRG
Allscheid, Robyn	National Corn Growers Association
Anderson, Sarah	Iowa State University
Andorf, Carson	USDA-ARS (MaizeGDB)
Angelovici, Ruthie	University of Missouri
Ansaf, Huda	University of Missouri-Columbia
Antonette Mercado, Mae	University of Illinois Urbana-Champaign
Aragon-Raygoza, Alejandro	North Carolina State University
Archontoulis, Sotirios	Iowa State University
Armstrong, Charles	Plastomics, Inc.
Ashraf, Arif	University of Massachusetts Amherst

Participant	Organization
Awale, Prameela	University of Missouri, Columbia
Azanu, Mercy	Iowa State University
Balint-Kurti, Peter	USDA-ARS, NC State University
Bang, Sohyun	University of Georgia
Bapat, Amruta	Iowa State University
Barl, Larissa	Technical University Munich
Barnes, Allison	North Carolina State University
Barreto Ortiz, Joan	University of Minnesota
Base, Hwan-Hee	Dept. of Central Area Crop Science, NICS, Rural Dev. Administration
Bass, Hank	Florida State University
Bennetzen, Jeff	University of Georgia
Bernau, Vivian	USDA-ARS Plant Introduction
Bertolini, Edoardo	Donald Danforth Plant Science Center
Berube, Benjamin	Ohalo Genetics
Best, Norman	USDA-ARS
Birchler, James	University of Missouri
Bock, Ralph	Max Planck Institute of Molecular Plant Physiology
Bohn, Martin	University of Illinois at Urbana-Champaign
Bonney Arku, Comfort	University of Massachusetts, Amherst

Participant	Organization
Bouwens, Brooke	Manchester University
Brady, Meghan	University of Georgia
Branch, Carl	University of Wisconsin-Madison
Braud, Max	Donald Danforth Plant Science Center
Braun, David	University of Missouri
Braynen, Janeen	Cold Spring Harbor Laboratory
Briggs, Steven	University of California San Diego
Brohammer, Alex	Bayer Crop Science
Bruce, Wesley	BASF
Bryan, Kempton	University of Georgia
Buckler, Edward	USDA-ARS, Cornell University
Burns, Michael	University of Minnesota
Busche, Michael	University of Wisconsin Madison
Butoto, Eric	North Carolina State University
Cahn, Jonathan	Cold Spring Harbor Laboratory
Camila Godoy dos Santos, Jenifer	University of Wisconsin Madison
Campbell, Darwin	Iowa State University
Cannon, Ethy	USDA-ARS (MaizeGDB)
Catlin, Nathan	Michigan State University

Participant	Organization
Chamberlain-Irwin, Heather	Iowa State University
Charcosset, Alain	INRAE
Chaudhry, Amina	Rutgers University
Chen, Yu-Ru	Iowa State University
Chen, Zongliang	Rutgers University
Chen, Keting	Iowa State University
Chen, Jeff	The University of Texas at Austin
Chin-Quee, Alec	University of Florida
Cho, Michelle	Washington University in St. Louis
Chomet, Paul	Chomet Consulting LLC
Clark, Jan	University of North Dakota
Clarke, Taylor	Stanford University
Clore, William	Iowa State University
Collier, Ray	UW Madison Crop Innovation Center
Cooper, Julian	University of Minnesota
Costa-Neto, Germano	Cornell University
Costich, Denise	Cornell University
Coursol, Sylvie	INRAE
Cowling, Craig	Iowa State University

Participant	Organization
Cryan, Elli	UC Davis
D Nagy, Ervin	Bayer Crop Science
Daniel Iohannes, Sessen	Cold Spring Harbor Laboratory
Dao, Linda	University of Florida
Dash, Linkan	Iowa State University
Davis, Danielle	University of Missouri - Columbia
Davis, Jensina	University of Nebraska-Lincoln
Davis, Ian	Bayer Crop Science
Dawe, Kelly	University of Georgia
de La Bretonne, Laurence	University of Wisconsin Madison
de Leon Gatti, Natalia	University of Wisconsin-Madison
de Neve, Amber	University of Massachusetts Amherst
De Schepper, Veerle	Inari
Debastiani Benato, Betina	Technische Universitat Munchen
Della Coletta, Rafael	University of Minnesota
DeTemple, Joseph	Iowa State University
Dhamrait, Natasha	University of California Davis
Dhungana, Singha	University of Missouri-Columbia
Dilkes, Brian	Purdue University

Participant	Organization
Dominguez, Liz	University of Illinois Urbana-Champaign
Draves, Melissa	University of Missouri
Dresselhaus, Thomas	University of Regensburg
Duong, Ha	University of Missouri - Columbia
Durst, Leah	Michigan State University
Eddy, Cassandra	University of Missouri
Egesa, Andrew	University of Florida
Egger, Rachel	Syngenta
El-Walid, Mohamed	Cornell University
Elyse Boris Alladassi, Mahule	Iowa State University
Epstein, Ruth	Cornell University
Ertl, David	Iowa Corn Growers Association
Eudy, Douglas	Bayer Crop Science
Evans, Matt	Carnegie Institution for Science
Evans, Lukas	Cornell University
Eve Ragas, Richie	Cornell University
Eveland, Andrea	Donald Danforth Plant Science Center
Facette, Michelle	University of Massachusetts, Amherst
Fakude, Mercy	Iowa State University

Participant	Organization
Fattel, Leila	Iowa State University
Ferris, Tyler	University of Nebraska-Lincoln
Filiault, Daniele	Bayer Crop Science
Finnegan, Colin	Iowa State University
Fitzgerald, Elizabeth	Cornell University
Flint-Garcia, Sherry	USDA-ARS
Foster, Tyler	Iowa State University
Fowler, John	Oregon State University
Fromm, Mike	epicrop
Gage, Joseph	NCSU
Gallavotti, Andrea	Waksman Institute, Rutgers University
Gardiner, Jack	University of Missouri
Geadelmann, Lander	Iowa State University
Gent, Jonathan	University of Georgia
Ghenov, Fernanda	University of Hawai'i at Manoa
Gilbert, Amanda	University of Minnesota
Gladman, Nicholas	USDA-ARS
Glover, Zehta	Florida State University
Gomez Cano, Lina	Michigan State University

Participant	Organization
Gosa, Sanbon	University of Illinois at Urbana-Champaign
Goudinho Viana, Willian	Stanford University
Gray, Katherine	University of Florida
Gray, Stephen	Iowa State University
Gregory, Jason	Rutgers University
Grieser, Elena	Iowa State University
Griffin, Brianna	Iowa State University
Grotewold, Erich	Michigan State University
Guan, Jiahn-Chou	University of Florida
Guerrero, Cristina	CINVESTAV - LANGEBIO
Guo, Yuxiang	Iowa State University
Gustin, Jeff	Maize Genetics Stock Center USDA-ARS
Hale, Charlie	Cornell University
Hall, Garret	University of Missouri - Columbia
Hampson, Ella	University of Hawaii
Hannah, Larkin	University og Florida
Hartwig, Thomas	Max Planck Institut for Plant Breeding
Hauge, Brian	TechAccel
Hayford, Rita	MaizeGDB

Participant	Organization
Hazelwood, Olivia	University of Delaware
Hein, Kirsten	Colorado State University
Helm, Matthew	USDA-ARS
Herr, Samuel	Cornell University
Hersh, Hope	University of Florida
Hickman, Jesse	Iowa State University
Higa, Lauren	University of Hawaii at Manoa
Higgins, Kaitlin	Iowa State University
Hinrichsen, Jake	Iowa State University
HIrsch, Candice	University of Minnesota
Hodge, John	University of Illinois Urbana-Champaign
Holding, David	University of Nebraska
Holland, James	USDA
Hollick, Jay	The Ohio State University
Hsu, Sheng-Kai	Institute for Genomic Diversity, Cornell University
Hu, Haixiao	UC Davis
Hubbard, Allen	Donald Danforth Plant Science Center
Hudson, Asher	North Carolina State University
Hufford, Matthew	Iowa State University

Participant	Organization
Hunter, Charles	USDA Agricultural Research Service
Hurst, Preston	University of Nebraska - Lincoln
Ikiriko, Irene	University of Delaware
Jackson, David	Cold Spring Harbor Laboratory
Jaiswal, Namrata	Agronomy, USDA-ARS
Jamann, Tiffany	University of Illinois Urbana-Champaign
Janik, Magdalena	Washington University-St. Louis
Jensen, Sarah	Syngenta
Ji, Zhongjie	Michigan State University
Ji, Jessica	Iowa State University
Jia, Hongwu	KWS
Jiang, Nan	Michigan State University
Jin, Hongyu	University of Nebraska-Lincoln
Johnson, Quinn	Cornell University
Johnston, Andrea	Donald Danforth Plant Science Center
Jones, Todd	Corteva Agriscience
Jr Garrido, Rommel	University of Minnesota
Julius, Benjamin	Bayer Crop Science
Kaeppler, Heidi	University of Wisconsin-Madison

Participant	Organization
Kaeppler, Shawn	University of Wisconsin-Madison
Kamei, Thanduanlung	University of Delaware
Kang, Minjeong	Iowa State University
Kanizay, Lisa	Bayer Crop Science
Kanna Mathivanan, Ramesh	University of Nebraska, Lincoln
Kappelmann, Kiara	Iowa State University
Kaur, Amanpreet	Purdue University
Kedar Marathe, Sarika	UW-Madison
Kelliher, Timothy	Syngenta Seeds
Kellogg, Elizabeth	Donald Danforth Plant Science Center
Kelly, Terice	University of Massachusetts, amherst
Kelly, Jacob	University of Missouri
Kent, Angela	University of Illinois at Urbana-Champaign
Khaipho-Burch, Merritt	Cornell University
Khangura, Rajdeep	Purdue University
Kick, Daniel	USDA-ARS
Kim, Saet-Byul	University of Nebraska-Lincoln
King, Kyle	Iowa State University
King, Joy	Bayer

Participant	Organization
Kinney, Shelly	Iowa State University
Klein, Stephanie	Iowa State University
Kloiber-Maitz, Monika	KWS Saat
Koch, Karen	University of Florida
Korth, Nate	University of Nebraska-Lincoln
Kosfeld, Tim	Donald Danforth Plant Science Center
Kuhn, Emily	Purdue University
Kumar, Rohit	Clemson University
Kunduru, Bharath	Clemson University
Kusmec, Aaron	Iowa State University
Lamb, Jonathan	Bayer Crop Sciences
Lane, Madison	Iowa State University
Langer, Matthias	Institute of Plant Genetics and Crop Plant Research (IPK)
Larson, Chris	University of North Dakota
Laspisa, Daniel	University of Georgia
Laurent, Maxime	UCLouvain
Lawrence-Dill, Carolyn	Iowa State University
Lee, Keunsub	Iowa State University

Participant	Organization
Leiboff, Samuel	Oregon State University
Lesko, Tyler	Penn State University
Lewis, Scott	Danforth Plant Science Center
Li, Mowei	The Ohio State University
Li, Catherine	University of Illinois
Li, Yawei	Iowa state university
Li, Fangyi	University of Nebraska-Lincoln
Li, Forrest	UC Davis
Li, Dongdong	Department of Agronomy, Iowa State University
Li, Xianran	USDA-ARS
LI, Hong	Bayer
Lima, Dayane	University of Wisconsin-Madison
Linders, Kyle	University of Nebraska-Lincoln
Lindsay, Penelope	Cold Spring Harbor Laboratory
Lisch, Damon	Purdue University
Liu, Alexander	Donald Danforth Plant Science Center, Washington University in Saint Louis
Liu, Hui	University of Florida
Liu, Zong-Yan	Cornell University
Liu, Peng	Donald Danforth Plant Science Center

Participant	Organization
Liu, Sanzhen	Kansas State University
Lloyd, John	Bayer Crop Science
Lopez-Corona, Lina	University of Nebraska Lincoln
Lor, Vai	University of Wisconsin-Madison
Lu, Zhenyuan	Cold Spring Harbor Laboratory
Lubberstedt, Thomas	Iowa State University
Lubkowitz, Mark	Saint Michael's College
Luebbert, Collin	Donald Danforth Plant Science Center
Lynn, Jason	Cold Spring Harbor Laboratory
Makarevitch, Irina	Hamline University, Biology Department
Man Tamang, Tej	Kansas State University
Manchego, Jordan	Michigan State University
Mankar, Sumeet	Danforth Plant Science Center
Marcela Escamilla Sanchez, Diana	Iowa State University
Marchant, Daniel	Stanford University
Martienssen, Rob	Cold Spring Harbor Laboratory
Martinez, Stephanie	University of California, Riverside
Matthes, Michaela	University of Bonn
McCarty, Donald	University of Florida

Participant	Organization
McCaw, Morgan	Ohalo Genetics
McGregor, Madison	Donald Danforth Plant Science Center
McGuire, Maddie	Ohalo Genetics
McSteen, Paula	University of Missouri
Meeley, Robert	DuPont-Pioneer (retired)
Mejia, Sendi	Purdue University
Meldau, Stefan	KWS SAAT SE & Co. KGaA
Menard, Claire	University of Minnesota
Mendieta, John	University of Georgia
Mertz, Rachel	Inari Agriculture
Meyers, Blake	Danforth Plant Science Center
Mickelson-Young, Leigh	NC State University
Mishra, Divya	University of Wisconsin
Mitchell, Madison	University of Missouri
Mondin, Mateus	"Luiz de Queiroz" College of Agriculture - ESALQ - University of Sao Paulo
Monier, Brandon	Cornell Univeristy
Moose, Steve	University of Illinois
Morais de Sousa Tinoco, Sylvia	Embrapa Maize and Sorghum
Motolai, Gergely	Kansas State University

Participant	Organization
Mourad Silva, Nadia	University of Florida
Mullens, Alex	University of Illinois
Munoz Javier, Rodrigo	CINVESTAV UGA-LANGEBIO
Mural, Ravi	Department of Agronomy and Horticulture, University of Nebraska Lincoln
Murguia, Simone	University of Hawaii at Manoa
Murphy, Katherine	Donald Danforth Plant Science Center
Muszynski, Michael	University of Hawaii at Manoa
Nandan Rai, Maruti	University of Illinois at Urbana Champaign
Negus, Karlene	Iowa State University
Nelissen, Hilde	VIB/UGent
Newton, Kathleen	University of Missouri
Ngara, Blessing	Iowa State University
Nicole Uy, Jaclyn	University of Hawaii
Niyonshuti Mizero, Olivier	University of Nebraska - Lincoln
Niyorukundo, Jonathan	University of Nebraska - Lincoln
Nystrom, Grace	University of Illinois
O'Connor, Lily	Danforth Plant Science Center
O'Connor, Devin	Pairwise
Ohlson, Erik	USDA-ARS

Participant	Organization
Oliver, Sarah	University of Missouri - Columbia
Olmedo Pico, Lia	Purdue University
Oren, Elad	Cornell University
Pandey, Piyush	Plant Genetics Research Unit, USDA-ARS
Panek, Bryan	Penn State University
Pardo, Anna	Michigan State University
Pawlowski, Wojtek	Cornell University
Peek, Kevin	University of Amsterdam
Peixoto, Marco	University of Florida
Peng, Mingsheng	Bayer
Peng, Jingyu	Bayer Crop Science
Phillips, Ronald	University of Minnesota
Pil, Hannah	North Carolina State University
Piri, Rebecca	University of Georgia
Portwood, John	USDA-ARS (MaizeGDB)
Prakash Chintamanani, Satya	Syngenta Seeds
Prasanth Durairaj Rajeswari, Jyothi	Iowa State University
Qais, Muhammad	Division of Plant Breeding Methodology
Read, Andrew	University of Minnesota

Participant	Organization
Rellan Alvarez, Ruben	North Carolina State University
Rhee, Sue	Carnegie Institution for Science
Rispress, Kimberly	East Carolina University
Roberts, Jason	University of Missouri, Columbia
Rocheford, Torbert	Purdue University
Rodene, Eric	University of Nebraska-Lincoln
Rolletschek, Hardy	IPK
Romay, Cinta	Cornell University
Ross-Ibarra, Jeffrey	University of California
Roziere, Julien	INRAE
Ruggiero, Diana	Oregon State University
Ruiz, Alejo	Iowa State University
Ruiz Vera, Ursula	Bayer Crop Sciences
Runyon, Matthew	University of Illinois
Sachs, Marty	Maize Genetics Cooperation - Stock Center, USDA-ARS
Sam Go, Young	Dept. of Central Area Crop Science, NICS, Rural Dev. Administration
Sanclemente, Maria-Angelica	Utrecht University
Santana, Alice	Iowa State University
Sara Akram, Hafiza	Florida State University

Participant	Organization
Scanlon, Michael	Cornell University
Schmelz, Eric	UC San Diego
Schmidt, Sophia	Iowa State University
Schmitz, Robert	University Of Georgia
Schnable, James	University of Nebraska-Lincoln
Schoemaker, Dylan	University of Wisconsin Madison
Schoenbaum, Greg	Iowa State University
Schulz, Aimee	Cornell University
Schumacher, Ally	Michigan State University
Scott, Paul	USDA-ARS
Searl, John	University of Wisconsin-Madison
Seetharam, Arun	Iowa State University
Selby, Rebecca	Syngenta
Sellers, Mitch	Donald Danforth Plant Science Center
Sen, Shatabdi	Iowa State University
Sheridan, Bianca	Florida State University
Sheridan, William	University of North Dakota - Biology
Shrestha, Nikee	University of Nebraska, Lincoln

Participant	Organization
Shrote, Robert	Michigan State University
Sidberry, Grace	University of Missouri
Sigmon, Brandi	University of Nebraska-Lincoln
Simmons, Tricia	ConferenceDirect
Singh, Ayush	Florida State University
Singh, Raksha	USDA-ARS
Singh, Anuradha	Michigan State University
Singh Brar, Manwinder	Clemson University
Sitar, Sidney	Michigan State University
Siu, Anson	University of Wisconsin Madison
Slewinski, Thomas	Bayer Crop Science
Slotkin, Keith	Danforth Plant Science Center / University of Missouri
Smith, Jada	University of Missouri
Snodgrass, Samantha	Iowa State University
Sosso, Davide	X the Moonshot Factory
Sparks, Erin	University of Delaware
Springer, Nathan	Bayer Crop Sciences
Stam, Maike	University of Amsterdam
Stewart, Nathan	Kansas State University

Participant	Organization
Stitzer, Michelle	Cornell University
Stokes, Ruthie	North Carolina State University
Strable, Josh	North Carolina State University
Strait, Peri	Ohalo Genetics
Strayhorn, Taylor	University of Georgia
Studer, Anthony	University of Illinois
Sullivan, Madsen	University of Illinois
Suzuki, Masaharu	University of Florida
Sweet, Dorothy	University of Minnesota
Sweet, Patrick	Syngenta
Sylvester, Anne	Marine Biological Laboratory
Tan, Bao-Cai	Shandong University
Tandukar, Nirwan	North Carolina State University
Taramino, Graziana	Bayer
Tassinari, Alberto	University of Bologna
Telleria Marloth, Janik	University Halle-Wittenberg
Teng, Chong	Danforth Plant Science Center
Thies, August	Donald Danforth Plant Science Center & University of Missouri
Thomas, Ty	University

Participant	Organization
Thompson, Addie	Michigan State University
Thompson, Beth	East Carolina University
Tian, Ran	Texas Tech University, Institute for Genomics of Crop Abiotic Stress Tolerance
Timmermans, Marja	Tuebingen University
Tong, Jingjing	University of Delaware
Topp, Christopher	Donald Danforth Plant Science Center
Torres, Hector	Stanford University
Torres, Vladimir	University of Nebraska-Lincoln
Townsend, Ella	Iowa State University
Tracy, William	University of Wisconsin-Madison
Tran, Alexandria	University of Illinois: Urbana-Champaign
Tran, Thu	Cold Spring Harbor Laboratory
Traylor, Zachary	University of Missouri-Columbia
Trifunovic, Slobodan	Bayer Company
Trost, Elysse	Iowa State University
Turkus, Jonathan	University of Nebraska-Lincoln
Turner, Sarah	Bayer Crop Science
Twohey, Robert	University of Illinois
Tyson, Destiny	North Carolina State University

Participant	Organization
Uberti, Alison	Federal University of ViѴßosa
Ullagaddi, Chidanand	University of Nebraska Lincoln
Ulutas, Musa	University of Nebraska-Lincoln
Uyehara, Aimee	University of California, Riverside
Vanous, Adam	USDA-ARS Plant Introduction
Varela, Jose	University of Wisconsin-Madison
Vollbrecht, Erik	Iowa State University
Vyn, Tony	Purdue University
Wagner, Ruth	Bayer
Walbot, Virginia	Stanford University emerita
Wallace, Jason	The University of Georgia
Walter, Nathalie	University of Wisconsin-Madison
Wang, Mingyu	university of georgia
Wang, Lin	Bayer Crop Science
Wang, Kan	Iowa State University
Ware, Doreen	USDA-ARS
Washburn, Jacob	USDA-ARS
Wear, Emily	NC State University

Participant	Organization
Webster, Brandon	Michigan State University
Weil, Clifford	National Scinece Foundation (Emeritus at Purdue University)
Wendt, Matthew	Iowa State University
Wheeler, Emily	NC State University
Whipple, Clinton	Brigham Young University
White, Mike	LIDEA
Whitt, Lauren	Danforth Plant Science Center
Wilburn, Colin	University of Missouri-Columbia
Wilson, McKena	Michigan State University
Winkelman, Dirk	Iowa State University
Wolters, Petra	Corteva Agriscience
Wong, Kong	Donald Danforth Plant Science Center
Woodhouse, Margaret	USDA-ARS (MaizeGDB)
Woods, Patrick	Colorado State University
Wrightsman, Travis	Cornell University
Wu, Hao	Cornell University
Xavier, Alencar	Corteva Agrisciences (1), Purdue University (2)
Xiao, Yuguo	Donald Danforth Plant Science Center

Participant	Organization
Xu, Xiaosa	Cold Spring Harbor Laboratory
Xu, Gen	University of Nebraska-Lincoln
Yanarella, Colleen	Iowa State University
Yandeau-Nelson, Marna	Iowa State University
Yang, Tianxiao	University of Florida
Yang, Zhikai	University of Nebraska-Lincoln
Yang, Hailong	The University of Massachusetts Amherst
Yang, Jinliang	University of Nebraska-Lincoln
Yang, Hua	University of Missouri-Columbia
Yi, Gibum	Chungnam National University
Yu, Jianming	Iowa State University
Zavala, MariaElena	CSU Northridge
Zebosi, Brian	Iowa State University
Zelkowski, Mateusz	Cornell university
Zeng, Yibing	University of Georgia
Zhai, Jingjing	Cornell University
Zhan, Junpeng	Donald Danforth Plant Science Center
Zhang, Xia	Syngenta
Zhao, Han	Jiangsu Academy of Agricultural Sciences

Participant	Organization
Zhou, Yan	Iowa State University
Zimmerman, David	University of Illinois
Zinselmeier, Matthew	X Company
Zinselmeier, Chris	Syngenta

History of the Maize Genetics Conference

Year	Annual	Location	Dates	Chair
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
1980	22	Allerton Park, IL	March 8-9	Earl Patterson

Year	Annual	Location	Dates	Chair
1979	21	Allerton Park, IL	March 10-11	Earl Patterson
1978	20	Allerton Park, IL	March 11-12	Earl Patterson
1977	19	Allerton Park, IL	March 12-13	Earl Patterson
1976	18	Allerton Park, IL	March 13-14	Earl Patterson
1975	17	Allerton Park, IL	March 8-9	Earl Patterson
1974	16	Allerton Park, IL	March 9-10	Earl Patterson
1973	15	Allerton Park, IL	March 10-11	Earl Patterson
1972	14	Allerton Park, IL	March 11-12	Earl Patterson
1971	13	Allerton Park, IL	March 13-14	Earl Patterson
1970	12	Allerton Park, IL	March 14-15	Earl Patterson
1969	11	Allerton Park, IL	March 15-16	Earl Patterson
1968	10	Allerton Park, IL	March 16-17	Earl Patterson
1967	9	Allerton Park, IL	March 11-12	Earl Patterson
1966	8	Allerton Park, IL	March 12-13	Earl Patterson
1965	7	Allerton Park, IL	March 13-14	Earl Patterson
1964	6	Allerton Park, IL	March 14-15	Earl Patterson
1963	5	Allerton Park, IL	March 9-10	Earl Patterson
1962	4	Allerton Park, IL	March 17-18	Earl Patterson
1961	3	Allerton Park, IL	March 18-19	Earl Patterson
1960	2	Allerton Park, IL	March 12-13	Earl Patterson
1959	1	Allerton Park, IL	January 8-9	John Laughnan, Ed Coe, Gerry Neuffer, and Earl Patterson

Notes

Notes

Notes

This conference received financial support from:

National Science Foundation
Bayer
Corteva Agriscience
Syngenta
Resolve BioSciences
KWS SAAT AG
NCGA
BASF
Inari
The Plant Cell
Power Pollen
Genetics Society of America

We thank these contributors for their generosity!

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

USDA ARS
Agricultural Research Service