60th Annual Maize Genetics Conference

Program and Abstracts

March 22 – March 25, 2018

Palais du Grand Large
Saint-Malo, France

This conference received financial support from:

National Science Foundation
ANR/Amaizing
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AGPM Maiz'Europ	BASF Plant Science
Agro Paris Tech	Caussade semences
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Biogemma	Euralis
Limagrain	Maïsadour
Promaïs	Benson Hill

We thank these contributors for their generosity!

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Cover image description

Male inflorescence (tassel) of the maize inbred line B73 at anthesis shedding pollen grains.

Cover art by

Lele Wang
University of Regensburg,
Germany

General Information

Meeting Registration

Thursday: 12:30 PM to 10:00 PM: Main Lobby Duguay Trouin

Friday: 8:00AM to 1:00 PM: Main Lobby Duguay Trouin

Meals

All meals will be served plated in the Grand Large room, level 1; serving hours as listed in the Program. Coffee, tea, soft drinks and snacks are available at no charge during the breaks that will be held in Poster rooms (levels 2 & 3) and J. Cartier rotunda, level 1. Please wear your nametag as that will give you entrance into the facility as well as the meals.

Talks and Posters

All Talks will be presented in the Chateaubriand auditorium, level 0.
Posters will be presented in the Lamennais room, level 3 and Surcouf rotunda, level 2. 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 numbered* posters are asked to stand by their posters 2:00-3:10 PM on Friday and 3:15-4:30 PM on Saturday. Presenters of *even numbered* posters should stand by their posters 3:10-4:15 PM on Friday and 2:00-3:15 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The maize meeting is a forum for presentation and discussion of unpublished material.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**. Posting information about talks and posters on social media is allowed **<u>only if</u>** the author has explicitly given permission to do so, which is indicated by the symbol in the abstract book.

Hospitality

After the evening sessions on Thursday, Friday, and Saturday there will be informal socializing and poster gazing in the Lamennais room, level 3 and Surcouf rotunda, level 2 with refreshments provided until 1 AM (3 free beverages will be offered each evening among a selection of soft drinks, regional beers and wine). Wine & cheese tasting at no charge will be organized on Friday evening. Additionally, on Saturday evening there will be music and dancing.
After 1 AM, the Bouvet room, level 1 will be available for continued socializing until 5 AM. This is a "private room" for socializing and professional networking, and it is permissible for alcoholic beverages to be brought in; however, you must stay in this room if you are carrying drinks, and please dispose of all trash and bottles in the room.

Steering Committee

Please share your suggestions and comments about the meeting with the 2018 Steering Committee

Alain Charcosset, Chair .... (alain.charcosset@moulon.inra.fr)
Mike Muszynski, co-Chair (mgmuszyn@hawaii.edu)
Erich Grotewold .....(grotewol@mail.msu.edu)
Karen McGinnis .....(mcginnis@bio.fsu.edu)
Jianbing Yan .....(yjianbing@gmail.com)
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Andrea Eveland .....(aeveland@danforthcenter.org)
Thomas Slewinski .....(thomas.l.slewinski@monsanto.com)
Andrea Gallavotti .....(agallavotti@waksman.rutgers.edu)
Stephen Novak .....(snnovak@dow.com)

Ex officio:
Carson Andorf
David Braun
Marty Sachs
Maud Tenaillon, Local Host

Acknowledgements

We warmly thank John Portwood and Carson Andorf for their tremendous efforts in organizing, assembling, and advertising the conference program. We are extremely grateful to Angela Freemyer and her team at the University of Missouri Conference Office and Rozenn Le Guyader at GQE-Le Moulon for helping to organize the conference, handling registration and dealing with a multitude of other issues. Special thanks are also extended to Sophie Fontaine, Aurélie Paris and Delphine Bru and their team at the Palais du Grand Large, for their help in organizing this conference, and to Darwin Campbell and John Portwood for providing AV and other support. Thanks go to Thomas Slewinski, Stephen Novak and Alain Charcosset for their efforts in securing funding to support graduate student attendance at this meeting. Finally, many, many thanks go to Maud Tenaillon for her work as local organizer and Marty Sachs for his wisdom in all things related to the Maize Meeting.

From the Maize Genetics Executive Committee:

Chair: Karen Koch 2020, Paul Chomet 2017, Sherry Flint-Garcia 2017, Shawn Kaeppler 2018, Patrick Schnable 2018, Kathy Newton 2019, Jianming Yu 2019, Natalia de Leon 2020, Tom Brutnell 2021, David Jackson 2021, and two new members to be announced.

Awards:

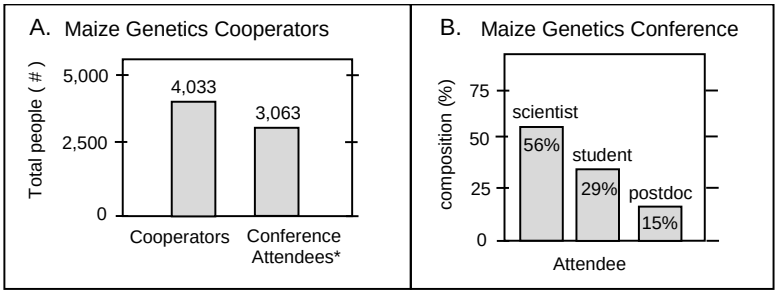
The Early Career Maize Genetics Award will be given to an individual that has been in a permanent position for less than 8 years. It is expected that the awardee will have made significant research contributions through genetic studies of maize or related species. (See MaizeGDB)
The 2018 Awardee is James Schnable at the University of Nebraska.

The Mid-Career Maize Genetics Award will be given to an individual that has been in a permanent position for 9-20 years. The winner will have an outstanding track record of discovery research in maize (or related species) genetics. (See MaizeGDB)
The 2018 Awardee is Mike Scanlon at Cornell University.

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

The Barbara McClintock Prize for Plant Genetics and Genome Studies has been created to memorialize the unequalled contributions of Dr. McClintock through providing recognition to the most outstanding plant geneticists of the present era. In memory of the many contributions of Dr. McClintock, this Prize will be awarded each year to one or more of the most creative minds and productive scientists in the study of plant genome structure, function and evolution, including the analysis of gene regulation and epigenetics. The 2018 Awardee is Rob Martienssen who will present the McClintock Prize Address. The 2019 Awardee will be announced at the meeting and will present the address next year (See MaizeGDB).

Defining the maize genetics community: Who are we?



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

NSF to fund future meetings and a Research Coordination Network for maize genetics:

The National Science Foundation has just notified the MGEC that support will be provided for a Research Coordination Network (RCN) entitled, "Broadening and Energizing the Maize Genetics Research Community." In addition to funding the next 5 years' Maize Genetics Conferences, the RCN will include separate workshops on 1) Databases and informatic tools, 2) Genetic resources and technologies, 3) Training, 4) Translating basic discoveries to commercial products, and 5) Broadening community diversity, expertise, and international interface. The RCN effort was led by former MGEC Chair, Shawn Kaeppler. Stay tuned for more information.

Planning for the coming year will be based on responses to the recent survey (from MGEC in February of this year). Some outstanding suggestions and data emerged. These, together with additional information on the RCN will be discussed at the Community Session of the Maize Genetics Conference this year and posted at MaizeGDB.

For those of you who provided the insightful replies to this year's survey – **Thank you!**

Let's Step Up Stewardship of Maize Data

Dear Maize Cooperators,

We are in an exciting time in biology with amazing new technologies and methods that lead to exponentially more data! We imagine a time in the near future when it will be easy to find all data for a gene, protein, or process with a few mouse clicks. But this will require changes in how we prepare, describe and make available our data. We at MaizeGDB have been working closely with representatives from 25 agriculturally related databases to craft standards for data consistency and sharing. The maize community has a real chance to lead in this effort. Let's all become excellent stewards of our data, and show others how good data management is done!

1. Put your sequences and genome assemblies in NCBI Databases.
NCBI (US), EBI (Europe), and DDBJ (Asia) provide stable, long-term databases for DNA, RNA and protein sequence data and create stable identifiers (accessions) for datasets. These three share sequence data on a daily basis. If your publication has any sequence data, make sure you submit it to NCBI, EBI, or DDBJ, and list the accession number in your paper. Note that SNP data will have to be submitted to EBI's EVA. If you need help with whole genome assembly submissions, contact MaizeGDB.

2. Don't rename genes that already have names.
Renaming genes that already have names is becoming a HUGE problem in maize, especially when an existing name is reused for a different gene. Please look up your gene at MaizeGDB before assigning a name, and follow the maize nomenclature guidelines. (https://www.maizegdb.org/nomenclature).

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

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

5. Budget time for Data Management.
Please budget time to do a good job of managing your data.

6. Familiarize yourself with the FAIR data sharing standards.
To support the reuse of scholarly data, a group of data scientists have created a set of recommendations to make data Findable, Accessible, Interoperable and Reusable.
https://www.force11.org/group/fairgroup/fairprinciples,

The MaizeGDB.org team
Contact us with any questions!

Useful Links

2018 Maize Meeting Website

http://maizegdb.org/maize_meeting/2018

2019 Maize Meeting Website (Available November 2018)

http://maizegdb.org/maize_meeting/2019

Abstract Book (Electronic version)

http://maizegdb.org/maize_meeting/abstracts/2018Program.pdf

Cover Image

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

The MaGNET Program and 2018 Awards

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

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

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

2018 MaGNET Awardees

Undergraduate

Estefania Aguilar-Gutierrez, California State University	Poster #178
Felicia Ebot-ojong, University of Georgia	
Tiffany Boynton, Florida Agricultural and Mechanical University	
Mohammed El-Walid, University of Missouri	Poster #148
Julia Owen, University of California Davis	
Husain Agha, University of Missouri	Poster #157
Brieana Hollis, Florida A&M University	

Graduate Student

Lamar Burton, Florida International University	
Maria-Angelica Sanclemente, University of Florida	Poster #46

Mentor Accompanying Student

Gokhan Hacisalihoglu, Florida A&M University	Poster #65
Rajandeep Sekhon, Clemson University	Poster #139
Lolita Adkins, University of California	
Kelly Swarts, Max Planck Institute for Developmental Biology	Talk #8
Arnaud Ronceret, Universidad Nacional Autónoma de México	Poster #144
Alexander Lipka, University of Illinois	
Ramesh Katam, Florida A&M University	Poster #125
Katy Guthrie, University of Missouri	Poster #135

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

Schedule of Events

Talks will be held in the Chateaubriand auditorium.
Posters will be displayed in the Lamennais room and Surcouf rotunda.

Thursday, March 22

1:30 PM – 5:00 PM	**OPTIONAL PRE-CONFERENCE WORKSHOPS**	
1:30 PM – 3:30 PM	**"Epigenetics"**	Maupertuis
1:30 PM – 3:30 PM	**"Maize tools & resources"**	Charcot
3:00 PM – 4:30 PM	**"Genetic transformation & editing"**	Chateaubriand
3:30 PM – 5:00 PM	**"Maize genomes"**	Maupertuis
	Pre-registration recommended for the above sessions.	
12:00 PM – 10:00 PM	**REGISTRATION** (Ground floor)	
3:00 PM – 6:00 PM	**POSTER HANGING** (Lamennais & Surcouf)	
6:00 PM – 7:15 PM	**DINNER** (Grand Large room)	
7:30 PM – 9:25 PM	**SESSION 1 – WELCOME / THE GENES THAT MAKE MAIZE** Chair: Alain Charcosset / Andrea Gallavotti Talks 1-5. Pages 24-28.	
7:30 PM	**WELCOME AND ANNOUNCEMENTS**	(Chateaubriand)
7:45 PM	**Thomas Widiez, INRA** *How to make maize seeds that look "not like dad": insights in double fertilization and prospects for novel breeding tools*	[T1]
8:05 PM	**Nina Chumak, University of Zürich** *Generating clonal progeny in maize*	[T2]
8:25 PM	**Thu Tran, University of Missouri - Columbia** *Maize Carbohydrate partitioning defective33 functions in sucrose export from leaves*	[T3]
8:45 PM	**Phillip Conklin, Cornell University** *NARROWSHEATH1 controls cell cycle dynamics to promote mediolateral outgrowth of leaf primordia*	[T4]
9:05 PM	**Keting Chen, Iowa State University** *Interrogating metabolic and transcriptomic networks to explain genetic, developmental and environmental variation in the cuticular lipid landscape on maize silks*	[T5]
9:30 PM – 1:00 AM	**INFORMAL POSTER VIEWING & HOSPITALITY** (Lamennais & Surcouf rotunda, Hospitality in Bouvet room until 5 am)	

Friday, March 23

8:00 AM – 1:00 PM	**REGISTRATION** (Ground floor)	
8:30 AM – 10:40 AM	**SESSION 2 – GENETIC ARCHITECTURE AND EVOLUTION** Chair: Jianbing Yan	Talks 6-11. Pages 29-34.
8:30 AM	**ANNOUNCEMENTS**	(Chateaubriand)
8:40 AM	**Markus Stetter, University of California - Davis** *How polygenic adaptation during domestication shaped the genetic architecture of maize*	[T6]
9:00 AM	**Christine Dillmann, Université Paris-Saclay** *Twenty years of divergent selection for flowering time from maize inbred lines*	[T7]
9:20 AM	**Kelly Swarts, Max Planck Institute** *Prehistoric selection for temperate climates has far-reaching implications for global germplasm*	[T8]
9:40 AM	**Aaron Kusmec, Iowa State University** *Distinct genetic architectures for phenotype means and plasticities in Zea mays*	[T9]
10:00 AM	**Jie Liu, Huazhong Agricultural University** *The conserved and unique genetic architecture of kernel size and weight in maize and rice*	[T10]
10:20 AM	**Jinyu Wang, Iowa State University** *Genome-wide nucleotide divergence and UV induced mutations following maize domestication*	[T11]
10:40 AM	**BREAK**	
11:10 AM – 12:55 PM	**SESSION 3 – PLENARY TALKS** Chair: Michael Muszynski	Pages 19 & 20.
11:10 AM	Introduction	(Chateaubriand)
11:15 AM	**Magnus Nordborg, Gregor Mendel Institute** *Epigenetic variation in Arabidopsis*	[PL1]
12:05 PM	**Francois Tardieu, INRA Montpellier** *Drought tolerance: which mechanisms, traits and alleles for which drought scenarios?*	[PL2]

Friday, March 23 (continued)

1:00 PM – 2:00 PM **LUNCH** (Grand Large room)

2:00 PM – 4:15 PM **POSTER SESSION 1** (Lamennais & Surcouf rotunda)

2:00 PM – 3:10 PM *Presenters should be at odd numbered posters.*

3:10 PM – 4:15 PM *Presenters should be at even numbered posters.*

Beverages will be available from 3:30 PM in Poster rooms & Jacques Cartier rotunda.

4:15 PM – 5:35 PM **SESSION 4 – EMERGING TOOLS AND CHALLENGES**
Chair: Erich Grotewold Talks 12-15. Pages 35-38.

4:15 PM **Kokulapalan Wimalanathan, Iowa State University** [T12]
Maize - GO Annotation Methods Evaluation and Review (Maize-GAMER)

4:35 PM **Mary Galli, Rutgers University** [T13]
Using DAP-seq to map genome-wide ARF transcription factor binding events in maize

4:55 PM **En Li, China Agricultural University** [T14]
Long-range interactions provide a topological basis for genetic regulation of complex traits in maize

5:15 PM **Samuel Leiboff, University of California - Berkeley** [T15]
The RNAseq Time Machine: Species-specific shifts in developmental timing and trajectory underlie morphological differences in maize tassel and sorghum panicle architecture

5:35 PM – 7:25 PM **SESSION 5 – PLENARY TALKS**
Chair: Alain Charcosset Pages 21 & 22.

5:35 PM Introduction (Chateaubriand)

5:45 PM **Ortrun Mittelsten Scheid, Gregor Mendel Institute** [PL3]
Light control of seed germination: the unusual role of usual suspects

6:35 PM **Jeff Ross-Ibarra, University of UC Davis** [PL4]
A bad genetic history of maize

7:30 PM – 8:45 PM **DINNER** (Grand Large room)

8:50 PM – 1:00 AM **INFORMAL POSTER VIEWING & HOSPITALITY**
(Wine & Cheese tasting in Lamennais & Surcouf rotunda, Jacques Cartier rotunda, Hospitality in Bouvet room until 5 am)

Saturday, March 24

8:30 AM – 10:30 AM	**SESSION 6 – INTERACTIONS WITH THE ENVIRONMENT** Chair: Natalia de Leon	Talks 16-21. Pages 39-44.
8:30 AM	**ANNOUNCEMENTS**	(Chateaubriand)
8:40 AM	**Sylvia Morais de Sousa, Embrapa Milho e Sorgo** *Enhancing phosphorus efficiency in maize and sorghum*	[T16]
9:00 AM	**Laurie Maistriaux, Université catholique de Louvain** *Regulation of aquaporin expression in maize: proximal and distal eQTLs*	[T17]
9:20 AM	**Justin Blancon, BIOGEMMA** *Innovative and high-throughput field phenotyping method provides leaf traits for breeding of drought tolerance - From Leaf Area Index dynamics to its physiological components*	[T18]
9:40 AM	**Joerg Degenhardt, Martin Luther University Halle** *Characterization of biosynthetic pathways and regulatory elements for the production of the volatile homoterpenes DMNT and TMTT in Zea mays*	[T19]
10:00 AM	**Marcel Bucher, University of Cologne** *Mycorrhizal phosphate uptake affects maize root-associated microbiota*	[T20]
10:20 AM	**Katherine Murphy, University of California - Davis** *Discovery of dolabralexins, previously unrecognized terpenoid defense compounds in maize (Zea mays).*	[T21]
10:40 AM	**BREAK**	
11:15 AM – 12:55 PM	**SESSION 7 – EXPRESSING THE GENOME** Chair: Andrea Eveland	Talks 22-26. Pages 45-49.
11:15 AM	**Jacob Washburn, Cornell University** *Predicting across the central dogma of molecular biology: DNA to mRNA abundance*	[T22]
11:35 AM	**Bradlee Nelms, Stanford University** *Mapping the archesporial cell to meiocyte progression using single-cell RNA-Seq*	[T23]
11:55 AM	**Jutta Baldauf, University of Bonn** *Single parent expression is a general mechanism driving extensive complementation of non-syntenic genes in maize hybrids*	[T24]
12:15 PM	**Edoardo Bertolini, Donald Danforth Plant Science Center** *The regulatory landscape of developing maize inflorescences: linking phenotypic variation to the functional non-coding genome*	[T25]

Saturday, March 24 (continued)

12:35 PM **Lu Chen, Huazhong Agricultural University** [T26]
Circular RNAs mediated by transposons are associated with transcriptomic and phenotypic variation in maize

1:00 PM – 2:00 PM **LUNCH** (Grand Large room)

2:00 PM – 4:30 PM **POSTER SESSION 1** (Lamennais & Surcouf rotunda)

2:00 PM – 3:15 PM *Presenters should be at even numbered posters.*

3:15 PM – 4:30 PM *Presenters should be at odd numbered posters.*

Beverages will be available from 3:30 PM in Poster rooms & Jacques Cartier rotunda.

4:30 PM – 5:30 PM **COMMUNITY SESSION - Maize Genetics Executive Committee**
MGEC Chair: Karen Koch (Chateaubriand)

5:30 PM – 7:30 PM **SESSION 8 – AWARDS AND MCCLINTOCK PRIZE PRESENTATION**
Chair: Karen Koch Page 23.

5:30 PM **Karen Koch, MGEC Chair**
Early- and Mid-Career Award, announcement of Emerson Award

5:45 PM **Paul Chomet, NRGene**
McClintock Prize Presentation

6:00 PM **Rob Martienssen, Cold Spring Harbor Laboratory**
Germline reprogramming, epigenetic inheritance and small RNA: how to avoid Bad Karma

6:50 PM- 7:50 PM Musical entertainment by Pao Bran and cocktails (Jacques Cartier rotunda)

8:00 PM – 9:50 PM **GALA DINNER** (Grand Large room)

10:00 PM – 1:00 AM **INFORMAL POSTER VIEWING/DANCE & HOSPITALITY**
(Lamennais & Surcouf rotunda, Music by Vincent Gaillard in Jacques Cartier rotunda, Hospitality in Bouvet room until 5 am)

2:00 AM Daylight Savings Time begins in EU. Change time to 3:00 AM.

Sunday, March 25

Posters should be taken down by 9 am!
TIME CHANGE!! Please remember in the EU daylight-savings time starts today: at 2:00 AM the time changes to 3:00 AM:

9:00 AM – 10:20 AM	**SESSION 9 – THE MAIZE (EPI)GENOMES** Chair: Maike Stam	Talks 27-30. Pages 50-53.
9:00 AM	**Daniel Grimanelli, Université de Montpellier** *Dynamics of DNA methylation during maize reproductive development*	[T27]
9:20 AM	**Sarah Anderson, University of Minnesota** *Transposable element contributions to the dynamic maize genome and transcriptome*	[T28]
9:40 AM	**Stephen Moose, University of Illinois** *The evolutionary dynamics of maize small RNAs reveals novel regulatory features in noncoding DNA*	[T29]
10:00 AM	**Adele Zhou, Cornell University** *Chromatin as a major determinant in the fate of meiotic double-strand breaks and positioning of COs along maize chromosomes*	[T30]
10:20 AM	**BREAK**	
10:50 AM – 12:00 PM	**SESSION 10 – COMMUNICATING WITHIN AND BETWEEN CELLS AND PLANTS** Chair: Thomas Slewinski	Talks 31-33. Pages 54-56.
10:50 AM	**Michael Muszynski, University of Hawaii** *Dissecting a new connection between cytokinin and jasmonic acid in control of leaf growth*	[T31]
11:10 AM	**Gerrit Beemster, University of Antwerp** *How grass keeps growing: a predictive model of leaf growth regulation based on studies in Zea mays*	[T32]
11:30 AM	**Hannes Claeys, Cold Spring Harbor Laboratory** *TREHALOSE-6-PHOSPHATE PHOSPHATASE4, a paralog of RAMOSA3, controls inflorescence architecture and shoot apical meristem activity in maize*	[T33]
11:50 PM	**CLOSING REMARKS**	
12:00 PM	**ADJOURNMENT**	

Posters

Computational and Large-Scale Biology

P1	**Joseph Gage** <jgage2@wisc.edu>	*An expanded diversity panel reveals the magnitude of the maize pan-genome and limitations of a single-reference genome sequence for GWAS*
P2	**Anne Lorant** <alorant@ucdavis.edu>	*Both hard and soft sweeps contribute to local adaptation of natural populations of teosintes*
P3	**Kokulapalan Wimalanathan** <kokul@iastate.edu>	*Bulked Segregant - genotyping-by-sequencing: Cost-effective and background independent genetic mapping of mutants and QTL*
P4	**Kelly Dawe** <kdawe@uga.edu>	*Coming soon: Whole genome assembly of the maize NAM founders*
P5	**Jerald Noble** <jnoble333@ufl.edu>	*Comparing alternative splicing in maize using 4 different reference genomes*
P6	**Ian Braun** <irbraun@iastate.edu>	*Computational classification of phenologs across biological diversity*
P7	**Johann Joets** <johann.joets@inra.fr>	*Deciphering molecular origin and functional impact of structural variation in maize through genome sequences comparison and integrative analysis of genetic variation, transcriptome and phenotype data.*
P8	**Eva Bauer** <e.bauer@tum.de>	*Four European Flint reference sequences complement the maize pan-genome*
P9	**Jiaqiang Dong** <jd1077@waksman.rutgers.edu>	*Functional analysis of maize kernel development*
P10	**Jan Freudenthal** <jan.freudenthal@uni-wuerzburg.de>	*Genomic prediction using TensorFlow*
P11	**Marcela Karey Tello-Ruiz** <mmonaco@cshl.edu>	*Gramene maize pan-genome browser*
P12	**Tessa Durham Brooks** <tessa.durhambrooks@doane.edu>	*Growth and metabolic responses of NAM parent lines to a brief exposure to cold during early seedling development*
P13	**John Fernandes** <john.fernandes@stanford.edu>	*High level analysis of W23 and A619 genomes (sequenced by Novogene) compared to B73 reference*
P14	**Jing Wang** <1964263754@qq.com>	*High resolution temporal and spatial transcription atlas of maize.*
P15	**Jin Cui** <juc326@psu.edu>	*Maize Ufo1 mutant plays a role in epigenetic regulation and alternative splicing*
P16	**Jesse Walsh** <jesse.walsh@ars.usda.gov>	*Functional divergence in maize subgenomes*
P17	**Ethalinda Cannon** <Ethy.Cannon@ars.usda.gov>	*MaizeGDB: stewardship for maize genome assemblies and annotation*
P18	**Jack Gardiner** <jack.m.gardiner@gmail.com>	*MaizeMine: a data mining warehouse for MaizeGDB*
P19	**Lisa Harper** <lisaharper@me.com>	*Making YOUR data and databases FAIR; Functional gene annotation and more!*
P20	**John Portwood** <john.portwood@ars.usda.gov>	*MaizeGDB: new resources for maize researchers*
P21	**Marcela Karey Tello-Ruiz** <mmonaco@cshl.edu>	*Mining maize with Gramene*

P22	**Savannah Savadel** <sds14d@my.fsu.edu>	*MOA (MNase Open Access) mapping: A new and efficient method for genome-wide open chromatin profiling in maize, demonstrated with developing earshoots*
P23	**Eve Wurtele** <mash@iastate.edu>	*New software to identify and explore the orphan genes of maize*
P24	**Georg Basler** <basler@mpimp-golm.mpg.de>	*Organ-specific maize metabolic models from ensemble modelling*
P25	**Xiang Gao** <caugxiang@163.com>	*Parallel transposase tagging (PTT-seq) technology is a cost-effective alternative for traditional Sanger sequencing*
P26	**Urte Schlüter** <u.schlueter@hhu.de>	*Predicting crop leaf parameter from leaf reflectance spectra*
P27	**Jun Zhao** <zhaojun01@caas.cn>	*Regulatory networks and kernel length related genes identified by eQTL analysis in 5 DAP maize kernels*
P28	**Lifang Zhang** <zhangl@cshl.edu>	*Regulatory Networks Governing Nitrogen Use Efficiencyt in Maize*
P29	**Johann Joets** <johann.joets@inra.fr>	*Sequence analysis of european maize inbred line F2 provides new insights into molecular and chromosomal characteristics of presence/absence variants.*
P30	**Jianing Liu** <jl03308@uga.edu>	*Sequence shattering and chromothripsis-like genome rearrangements following biolistic transformation in rice and maize*
P31	**A. Mark Settles** <settles@ufl.edu>	*Soil-based machine vision seedling emergence assay for studying cold tolerance in maize*
P32	**Patrick Monnahan** <pmonnaha@umn.edu>	*Structural variation analysis in the Wisconsin Diversity Panel using multiple de novo genome assemblies*
P33	**Hank Bass** <bass@bio.fsu.edu>	*The NUPRIME project: nuclease profiling of four reference tissues as a resource for maize epigenomics.*
P34	**Peter Bradbury** <pjb39@cornell.edu>	*The practical haplotype graph: using a simplified pan-genome to impute genotypes from skim sequence*
P35	**Junpeng Shi** <shijunpeng_cau@163.com>	*Tracing the heritability of agronomic traits in maize*
P36	**Christine Gault** <cg449@cornell.edu>	*Tripsacum de novo transcriptome assemblies reveal parallel gene evolution in maize and Tripsacum after ancient polyploidy*
P37	**Alexandra Asaro** <aasaro@wustl.edu>	*Using two parent genome mapping to identify expression level quantitative loci in maize roots*

Biochemical and Molecular Genetics

P38	**Bertrand Hirel** <bertrand.hirel@inra.fr>	*A system biology approach to identify biochemical markers representative of high yielding maize lines*
P39	**Peng Liu** <mcliup@ufl.edu>	*An opaque phenotype and altered mitochondrial respiratory chain are caused by a maize rug3 mutation*
P40	**Margaret Bezrutczyk** <Margaret.Bezrutczyk@hhu.de>	*Apoplasmic phloem loading in Zea mays L. requires three SWEET sucrose transporters*
P41	**Norman Best** <bestn@missouri.edu>	*barren stalk3 is required for axillary branch development and maps to the same location as barren stalk2.*
P42	**Viviane Cristina Heinzen da Silva** <vchsilva@gmail.com>	*Biochemical and structural characterization of a new maize receptor-like kinase likely involved in drought stress.*
P43	**Zhihong Lang** <langzhihong@caas.cn>	*Characterization and functional analysis of maize Terpene synthetase6 (TPS6) Gene*

P44	**David Braun** <braundm@missouri.edu>	*Cloning and characterization of a gene which disrupts carbohydrate partitioning in maize*
P45	**Nathan Swyers** <ncs89f@mail.missouri.edu>	*Development of an amenable system for site-specific addition to a maize B-Chromosome*
P46	**Maria-Angelica Sanclemente** <sanangelma@gmail.com>	*Effect of sugar levels on the low oxygen response of maize roots*
P47	**Lei Wang** <wanglei01@caas.cn>	*Effect of water-deficit on phenotypes and transcriptomes of developing tassel in maize*
P48	**Nick Ferrigno** <nferrigno@mail.smcvt.edu>	*Elucidating the transcriptional regulatory network controlling SUT and SWEET genes in maize*
P49	**Borrelli Marocco** <virginiamaria.borrelli@unicatt.it>	*Enhancement of maize disease resistance by CRISPR/Cas9 editing of LOX and WRKY genes*
P50	**Senlin Xiao** <forestxiao@genetics.ac.cn>	*Exploration of the molecular mechanism of CMS-S*
P51	**Britney Moss** <mossbl@whitman.edu>	*Functional characterization of maize auxin signaling modules in yeast*
P52	**Samuel Hokin** <shokin@carnegiescience.edu>	*GBS identification of genomic loci associated with embryo twins in ig1 mutants*
P53	**Isabelle Quilleré** <isabelle.quillere@inra.fr>	*Genetic variability for nitrate uptake in a core collection of European and American maize (Zea mays L.) lines.*
P54	**Xiaowen Shi** <shix@missouri.edu>	*Genome imbalance impacts global gene expression and small RNA expression in maize*
P55	**haiyang wang** <wanghaiyang@caas.cn>	*Genomic analyses of the genetic basis for shaping maize plant architecture for adapting to increased planting density*
P56	**Jacob Washburn** <jdw297@cornell.edu>	*Heritable differences in C4 photosynthetic sub-type across diverse maize germplasm indicate utility in discover and breeding*
P57	**Michiel Bontinck** <mibon@psb.ugent.be>	*High resolution interactomics in the maize leaf growth zone, using AN3 as a case-study*
P58	**Vincenzo Rossi** <Vincenzo.Rossi@crea.gov.it>	*INDETERMINATE1 direct targets and regulatory mechanisms*
P59	**Gibum Yi** <gibumyi@gmail.com>	*Investigating seed mineral composition of Korean landrace corns (Zea Mays L.)*
P60	**Heidi Kaeppler** <hfkaeppl@wisc.edu>	*Maize genomics, genetic engineering and gene editing research and services at the Wisconsin Crop Innovation Center*
P61	**Jacob Corll** <jbcorll123@gmail.com>	*Maize RNA Binding Motif Protein48 (RBM48) is required for minor intron splicing and promotes endosperm cell differentiation*
P62	**Claudiu Niculaes** <claudiu.niculaes@tum.de>	*Maximizing photosynthesis efficiency in maize*
P63	**Bing Liu** <bl472@cornell.edu>	*Meiotic recombination landscape in maize: impact of chromosome axis and heat stress*
P64	**Jing Wang** <1964263754@qq.com>	*Miniature seed 2109 encodes a nitrate transporter protein required for maize seed development.*
P65	**Gokhan Hacisalihoglu** <gokhan.h@famu.edu>	*Modulation of cold sensitivity and early seedling performance by priming in 27 maize NAM parental inbred lines*
P66	**Rentao Song** <rentaosong@cau.edu.cn>	*Opaque11 is a central hub of the regulatory network for maize endosperm development and nutrient metabolism*
P67	**Jennifer Arp** <jarp@danforthcenter.org>	*Phosphoenolpyruvate carboxykinase mutants reveal interaction of C_4 photosynthesis and nitrogen utilization in maize*
P68	**Giulia Castorina** <giulia.castorina@unimi.it>	*Regulation of cuticle deposition during juvenile vegetative phase in maize*

P69	**Uta Paszkowski** <up220@cam.ac.uk>	*Rhizosphere signaling in arbuscular mycorrhizal symbiosis of maize.*
P70	**Jon Cody** <joncody@mail.missouri.edu>	*Site specific recombinases: Tools for genetic engineering in maize*
P71	**Changsheng Li** <c.li3@uva.nl>	*Strigolactone biosynthesis in Zea mays (maize)*
P72	**Jun Yang** <yangjun@sibs.ac.cn>	*The maize Oxalyl-CoA Decarboxylase1 acting downstream of Opaque7 is required for oxalate degradation and central metabolism and affects seed nutritional quality*
P73	**John Gray** <john.gray5@utoledo.edu>	*The maize TFome and the GRASSIUS database: Resources for regulomics in the grasses*
P74	**Fadi El Hage** <fadi.el-hage@inra.fr>	*Tissues lignification, cell wall p-coumaroylation and degradability of maize stems depend on water status*
P75	**Marcel Baer** <Marcel.Baer@uni-bonn.de>	*Validation and functional characterization of the maize lateral rootless 1 (lrt1) gene*
P76	**Aleksej Abramov** <aleksej.abramov@tum.de>	*Why does maize produce benzoxazinoids and transgenic Arabidopsis not?*
P77	**Miaoyun Xu** <xumiaoyun@caas.cn>	*Zma-miR169q/NF-YA14 module is involved in salt stress tolerance in maize*
P78	**Chaobin Li** <chaobinli@cau.edu.cn>	*ZmbZIP22 is a new transcription factor for 27-kD γ-zein gene*

Cell and Developmental Biology

P79	**Francois Tardieu** <francois.tardieu@inra.fr>	*A phenomics-based dynamic model of growth and yield to simulate hundreds of maize hybrids in the diversity of European environments*
P80	**Wei Huang** <wilsonhuang23@cau.edu.cn>	*A point mutation in maize hzMS1 gene causes the meiosis defects in heterozygous mutants*
P81	**Tom Van Hautegem** <tohau@psb.vib-ugent.be>	*Analysis of leaf growth under mild drought in maize recombinant inbred lines.*
P82	**David Perez** <Dp15g@my.fsu.edu>	*Bioremediation analysis of water pollutants and pathogens within household water in rural south India*
P83	**Qiujie Liu** <qiujieliu@waksman.rutgers.edu>	*Characterization and cloning of needle1 (ndl1), a temperature sensitive mutant affecting reproductive organogenesis*
P84	**Kyle Swentowsky** <kws67291@uga.edu>	*Characterization of a novel Kinesin protein required for Ab10 meiotic drive*
P85	**Wenjing She** <wenjing.she@uzh.ch>	*Characterization of meiotic defects in the maize nrf4 mutant*
P86	**Edgar Demesa-Arevalo** <edemesaa@cshl.edu>	*Characterization of RAMOSA3 putative nuclear interactors and their role in inflorescence development in maize*
P87	**Gabriella Consonni** <gabriella.consonni@unimi.it>	*Characterization of the maize lil1-1 mutant defective in the Brassinosteroid C-6 oxidase*
P88	**Marschal Bellinger** <mbell008@ucr.edu>	*Characterizing the function of kinectin in plants via Zea mays and Arabidopsis thaliana*
P89	**Laurens Pauwels** <lapau@psb.vib-ugent.be>	*CRISPR/Cas9 gene editing in the maize inbred line B104*
P90	**Bénédicte Bakan** <benedicte.bakan@inra.fr>	*Deposition of lipids in starch granules of maize endosperm is closely related to zein synthesis and programmed cell death*
P91	**Kamila Kalinowska-Brandt** <kamila.kalinowska@ur.de>	*DiSUMO-LIKE interacts with cell cycle and RNA pathways, and regulates early embryo development in maize*

P92	**Travis Hattery** <thattery@iastate.edu>	*Diversity in the surface lipid composition of maize silks among Wisconsin Diversity Panel inbreds in two environments*
P93	**Yongrui Wu** <yrwu@sibs.ac.cn>	*early delayed kernel 1 (edk1) encodes a microtubule-located protein essential for early endosperm development in maize*
P94	**Angus Vajk** <vajking@berkeley.edu>	*fun is: pleiotropy, unknown genes and double mutants*
P95	**lele wang** <lele.wang@ur.de>	*Functional analysis of pollen-specific RALFs during reproduction in maize*
P96	**Jara Oppenheimer** <jara.oppenheimer@uni-hamburg.de>	*Functional analysis of the heterotrimeric Gγ subunits in maize*
P97	**Xingli Li** <xingli.li@ur.de>	*Functional characterization of male gametophyte genes under heat stress*
P98	**Chao Bian** <chao.bian@rutgers.edu>	*Functions of PP2A phosphatases in Arabidopsis stomatal development*
P99	**Beth Thompson** <thompsonb@ecu.edu>	*Genetic control of maize floral development*
P100	**Santiago Alvarez Prado** <santiago.alvarez-prado@inra.fr>	*Genetic control of stomatal conductance in maize and conditional effects to water deficit and evaporative demand as revealed by phenomics*
P101	**Dong Zhaobin** <dongz@berkeley.edu>	*Genetic mechanisms for bud suppression during maize domestication*
P102	**Xiaoli Ma** <xiaoli.ma@uni-tuebingen.de>	*Genome-wide analysis of small RNA-controlled gene networks in leaf development*
P103	**Claude Welcker** <claude.welcker@inra.fr>	*Grain abortion under drought in maize: expansive growth and hydraulics also matter*
P104	**Kevin Begcy** <kevin.begcy@ur.de>	*Heat stress induced male sterility during pollen development in maize*
P105	**Xiaolong Tian** <cautxl@163.com>	*Hetero-fertilization along with failed egg-sperm cell fusion reveals single fertilization involved in in vivo haploid induction in maize*
P106	**Laurine Gilles** <laurine.gilles@ens-lyon.fr>	*How to make maize seeds that look "not like dad": insights in double fertilization and prospects for novel breeding tools.*
P107	**Hardeep Gumber** <hardeep@bio.fsu.edu>	*Identification and characterization of LINC complex proteins in Zea mays L.*
P108	**Joke De Jaeger-Braet** <joke.jaeger-braet@uni-hamburg.de>	*Identification of the translational landscape of Arabidopsis and Maize meiocytes*
P109	**Yanfang Du** <yanfangdu@webmail.hzau.edu.cn>	*Integrated analysis of protein abundance, transcript level, and tissue diversity to reveal developmental regulation of maize*
P110	**Annis Richardson** <annisrichardson@berkeley.edu>	*Keeping Separate: mysterious boundaries and the grass leaf*
P111	**Muriel Longstaff** <mtlongstaff@gmail.com>	*Kinetic and morphological tiller meristem development in domesticated and wild Setaria*
P112	**Madelaine Bartlett** <mbartlett@bio.umass.edu>	*Leveraging next-generation sequencing technology for rapid gene cloning*
P113	**Ching-Chih Tseng** <tsengyin@gate.sinica.edu.tw>	*MAC1 and AM1 play critical but independent roles to regulate the mitosis-to-meiosis transition of pollen mother cells in maize*
P114	**Alyssa Anderson** <alyssa.amy@berkeley.edu>	*Maize genetics at the intersection of development and immunity*

P115 **Josh Strable** <jjs369@cornell.edu> — *Maize leaf primordia microdomains show genetic signatures of proximal-distal boundary patterning*

P116 **Robert Maple** <R.Maple@warwick.ac.uk> — *Male-specific argonaute (MAGO) proteins are necessary for meiosis in maize*

P117 **Fatma Aydinoglu** <faydinoglu@gtu.edu.tr> — *MicroRNAs targeting developmental transcription factors control maize leaf size by switching cell proliferation to cell expansion*

P118 **Lei Ding** <lei.ding@uclouvain.be> — *Modification of the expression of PIP2;5 plasma membrane aquaporin affects maize water relations and growth*

P119 **Mario Arteaga-Vazquez** <maarteaga@uv.mx> — *MOP1 regulates germline specification and gamete development in maize*

P120 **Yang-Seok Lee** <Y.Lee.6@warwick.ac.uk> — *Movement of premiotic phased small-interfering RNAs (phasiRNAs) is essential for male meiosis in maize*

P121 **Steffen Knauer** <steffen.knauer@uni-tuebingen.de> — *Natural variation in the molecular circuitry underlying cell type specification drives key plant architectural traits in maize*

P122 **Matthew Warman** <warmanma@oregonstate.edu> — *nop genes promote pollen tube growth in the maize male gametophyte*

P123 **Karina van der Linde** <karina.van-der-linde@ur.de> — *Pursuing maize (Zea mays) tassel development by small RNA sequencing, transcriptomics, and proteomics*

P124 **Max Braud** <mbraud@danforthcenter.org> — *Redundant roles of two paralogous INDETERMINATE DOMAIN (IDD) transcription factors in maize development*

P125 **Ramesh Katam** <ramesh.katam@famu.edu> — *Relative abundance of Proteins to Multiple stresses suggests Cross-Tolerance Mechanism in Soybean*

P126 **Didier Marion** <didier.marion@inra.fr> — *Responses to hypoxia and endoplasmic reticulum stress: relationships with vitreousness of maize endosperm*

P127 **Natalie Deans** <deans.11@osu.edu> — *RMR12 is a CHD3 nucleosome remodeler required for maintaining paramutations and normal development*

P128 **Oscar Sanz Mora** <osanz.osm@gmail.com> — *Role of introns in the correct expression of the meiotic cyclin SOLO DANCERS (SDS) of maize in A. thaliana*

P129 **Jack Satterlee** <jws429@cornell.edu> — *Search for genetic modifiers of knotted1*

P130 **China Lunde** <lundec@berkeley.edu> — *Sex and violence: the classic maize mutant Tasselseed5 is encoded by a wound-responsive JA-inactivating enzyme*

P131 **Jack Satterlee** <jws429@cornell.edu> — *Single Cell Transcriptomic Analysis of the Developing Maize Embryo*

P132 **Silvio Salvi** <silvio.salvi@unibo.it> — *The genetic control of a new tasselseed mutant in maize*

P133 **Michaela Matthes** <matthesm@missouri.edu> — *The role of boron in vegetative and reproductive development in maize*

P134 **Dave Stateczny** <dave.stateczny@uni-hamburg.de> — *The role of CT2 in maize internode development*

P135 **Katherine Guthrie** <klgdn2@mail.missouri.edu> — *The role of maize mutant Suppressor of sessile spikelets 2 (Sos2) in meristem maintenance*

P136 **Thomas Hughes** <thomas.hughes@plants.ox.ac.uk> — *The role of ZmSCR1 and ZmSCR1h during maize leaf development*

P137 **Janlo Robil** <jmrobil@mail.mizzou.edu> — *The scarecrow mutation enhances auxin-related leaf and inflorescence defects in maize*

P138 **Peng Yu** <yupeng@uni-bonn.de> — *Tissue- and cell-specific multi-omics analyses define a key molecular pathway of lateral root initiation and its interaction with arbuscular mycorrhizal fungi in maize*

P139 **Rajandeep Sekhon** <sekhon@clemson.edu> — *Towards a comprehensive understanding of genetic architecture underlying senescence*

P140 **Martina Balboni** <martina.balboni@uni-hamburg.de> — *Towards live imaging of meiosis in maize*

P141 **Matthew Warman** <warmanma@oregonstate.edu> — *Transcriptomic characterization of male sexual reproduction in maize*

P142 **Vasco Köhling** <vasco.koehling@gmx.de> — *Transient interaction analysis of the Zea mays (L.) CT2 and PLDα1 proteins in Nicotiana benthamiana (L.)*

Cytogenetics

P143 **Ding Hua Lee** <dinghua@gate.sinica.edu.tw> — *AFD1 and DSY2 are both required for chromosomal localization of ASY1 and meiotic double-strand break formation in maize*

P144 **Arnaud Ronceret** <ronceret@ibt.unam.mx> — *Analysis of ten meiotic mutants of maize*

P145 **Natalie Nannas** <njnannas@hamilton.edu> — *Dynamics of meiotic spindle assembly in Zea mays*

P146 **Jodi Weiss** <jdweiss@hamilton.edu> — *Frequency of abnormal chromosome 10 in tropical landraces of Zea mays*

P147 **Olivier Martin** <olivier.c.martin@inra.fr> — *Meiotic crossovers in maize: the interfering and non-interfering recombination pathways have different landscapes*

P148 **Mohamed El-Walid** <mze99d@mail.missouri.edu> — *Visualizing plastid sequences present on the B Chromosome of maize*

Education & Outreach

P149 **Irina Makarevitch** <imakarevitch01@hamline.edu> — *Doing genetics research in the classroom: CRISPR-Cas9 and cold stress in plants*

P150 **Josiane Lorgeou** <j.lorgeou@arvalis.fr> — *Evaluation of genetic progress in grain and silage corn afforded by the renewal of varieties in France over the past 30 years*

P151 **Francis Denisse McLean Rodriguez** <f.mcleanrodriguez@sssup.it> — *Fifty years of mirrored in situ and ex situ conservation of Mexican maize landraces: socioeconomic dynamics and genomic diversity*

P152 **Denise Costich** <d.costich@cgiar.org> — *Learning how to rescue a landrace: A study of the giant maize, Jala, and the community who grows it*

P153 **Denise Costich** <d.costich@cgiar.org> — *The maize collection of CIMMYT's germplasm bank: promoting the conservation, use and study of diversity*

P154 **Hyeyoung Lee** <leehye@missouri.edu> — *NSF Plant Transformation Workshop*

P155 **Cristina Marco** <marco@cshl.edu> — *Maize annotation jamboree on last B73 RefGen_V4 assembly*

Quantitative Genetics & Breeding

P156 **Lorenzo Stagnati** <lorenzo.stagnati@unicatt.it> — *A candidate gene approach to identify markers associated to Fusarium Kernel Rot resistance in maize*

P157 **Husain Agha** <hiagha@mail.missouri.edu> — *A Novel Mating Design Provides High Power to Detect Epistasis in Maize*

P158 **Clement Buet** <clement.buet@biogemma.com> — *A RNA-Seq atlas on the founder lines of the MAGIC population "BALANCE", a supplementary tool for gene discovery*

P159 **Matthieu Bogard** <m.bogard@arvalis.fr> — *Agronomic trials characterization and clustering using water and nitrogen stress indices simulated with a crop model*

P160 **Alain Charcosset** <alain.charcosset@inra.fr>
AMAIZING, a project on Maize Integrative Genomics supported by the French Investment for the Future Program

P161 **Jorge Nieto-Sotelo** <jorge.nieto@ib.unam.mx>
Analyses of genetic variation associated to deep planting resistance in maize revealed genes controlling development, growth, and adaptation to soil conditions

P162 **Clément Buet** <clement.buet@biogemma.com>
BALANCE, a powerful MAGIC population for the identification of genetic determinants involved in the variation of traits of interest in maize

P163 **Violeta Andjelkovic** <avioleta@mrizp.rs>
Changes in carotenoid and tocopherol content in maize grain during cold storage

P164 **Lucio Conti** <lucio.conti@unimi.it>
Co-regulation of ZCN8 and ZCN12 underlies Maize flowering variability

P165 **Randall Wisser** <rjw@udel.edu>
Computer-vision into the biology of fungal-leaf interactions in maize

P166 **Junjie Zou** <zoujunjie@caas.cn>
Construction of maize grain moisture content related near-isogenic lines for developing SNP molecular marker

P167 **Torsten Pook** <torsten.pook@uni-goettingen.de>
Creation of subgroup specific haplotype blocks and libraries

P168 **Arlyn Ackerman** <arlyna@clemson.edu>
Deployment of a novel phenotypic platform based on biomechanical engineering principles provides novel insights into stalk lodging in maize

P169 **Jianming Yu** <jmyu@iastate.edu>
Design Thinking and Data Mining in Plant Breeding

P170 **Clement Mabire** <clement.mabire@inra.fr>
Development of a new high throughput 105K presence/absence variation genotyping array for quantitative genetic studies

P171 **Rahime Cengiz** <rahime.cengiz@tarim.gov.tr>
Development of late temperate maternal haploid inducers

P172 **Adrienne Ressayre** <adrienne.ressayre@u-psud.fr>
Developmental and morphological changes associated to flowering time shifts produced during divergent selection experiments in maize : developmental transitions and architecture

P173 **Christine Dillmann** <christine.dillmann@inra.fr>
Developmental and morphological changes associated to flowering time shifts produced during divergent selection experiments in maize : phyllochron

P174 **Pedro Revilla** <previlla@mbg.csic.es>
Dissecting the genetics of cold tolerance in maize

P175 **Yaoyao Wu** <yyw_cau@163.com>
Dissecting the role of lncRNA during maze domestication

P176 **Brigitte Gouesnard** <brigitte.gouesnard@inra.fr>
Diversity analysis within a collection of 1191 flint maize inbred lines using genotyping-by-sequencing

P177 **Yacine Diaw** <yacine.diaw@supagro.fr>
Diversity of maize landraces from south-west of France: origin and morphological differentiation analyzes

P178 **Estefania Aguilar-Gutierrez** <Estefania55@mail.fresnostate.edu>
Does subspecific variation correspond to genetic or cytotypic variation in the widespread taxon Phlox speciosa (Polemoniaceae)?

P179 **Matteo Dell'Acqua** <m.dellacqua@santannapisa.it>
eQTL mapping and genome wide association study for maize leaf traits using markers derived by RNA sequencing of two RIL populations

P180 **Marlon Caicedo** <mcaicedo@mbg.csic.es>
Evaluation of functional stay-green in maize inbred lines and their relationship with agronomic traits

P181 **Laetitia Virlouvet** <laetitia.virlouvet@inra.fr>
Exploring the genetic basis of cell wall traits upon contrasted water regimes in maize

P182 **Tes Posekany** <posekany@iastate.edu>	*Fine mapping of metabolite-QTLs for extracellular surface lipid accumulation on maize silks*
P183 **Adam Vanous** <adamv@iastate.edu>	*Flowering time stability in doubled haploid lines derived from exotic maize*
P184 **Clement Buet** <clement.buet@biogemma.com>	*From the sequencing of 16 MAGIC population founders to a 8 million SNP resource on the BALANCE panel*
P185 **Julie Fievet** <julie.fievet@inra.fr>	*Genetic and molecular basis of maize hybrid vigor: genome wide association studies in factorial hybrid designs*
P186 **Renyu Zhang** <zhangrenyu@live.com>	*Genetic architecture of complex traits in a maize-teosinte population*
P187 **Kathryn Michel** <kathryn.michel@wisc.edu>	*Genetic dissection of plant height and flowering using two Stiff Stalk multi-parent advanced generation intercross populations of maize*
P188 **Nikola Grcic** <ngrcic@mrizp.rs>	*Genetic distance in relation with specific combining abilities and heterosis for vegetative traits in maize*
P189 **Eva Bauer** <e.bauer@tum.de>	*Genetic variation for early development and cold tolerance in DH libraries from maize landraces*
P190 **Jose Varela** <jvarela@wisc.edu>	*Genome wide association studies for kernel starch and protein content in the Wisconsin diversity (WiDiv) maize association panel*
P191 **Mélisande Blein-Nicolas** <melisande.blein-nicolas@inra.fr>	*Genome wide association study for protein expression under normal and water deficit conditions in maize leaves*
P192 **Jonas Rodriguez** <jrodriguez36@wisc.edu>	*Genome-Wide association of biomass digestibility in the Wisconsin diversity panel*
P193 **Torbert Rocheford** <torbert@purdue.edu>	*Genome-wide mapping of kernel color in maize (Zea Mays L.) reveals associations with isoprenoid and carotenoid biosynthesis genes and carotenoid degradation genes*
P194 **Stéphane Nicolas** <stephane.nicolas@inra.fr>	*Genome-wide SNP genotyping of DNA pools identifies original landraces to enrich maize breeding pools*
P195 **Albrecht E. Melchinger** <melchinger@uni-hohenheim.de>	*Genomic prediction within and among doubled-haploid libraries from maize landraces*
P196 **Dustin MacLean** <dmacle02@uoguelph.ca>	*Genomic regions associated with Goss's Wilt resistance in the commercial maize germplasm pool.*
P197 **Simon Rio** <simon.rio@inra.fr>	*Genomic selection efficiency and a priori estimation of accuracy in a structured dent maize panel*
P198 **Natalia Martinez-Ainsworth** <natalia.martinez@inra.fr>	*Genomic signatures of local adaptation unveil association with present phenotypic variation in teosintes*
P199 **Brigitte Gouesnard** <brigitte.gouesnard@inra.fr>	*Genotyping-by-sequencing highlights original diversity patterns within a European collection of 1191 maize flint lines, as compared to the maize USDA genebank*
P200 **Torbert Rocheford** <torbert@purdue.edu>	*Germplasm enhancement using former plant variety protected inbreds*
P201 **Milan stevanović** <mstevanovic@mrizp.rs>	*Grain yield and stability parameters of ZP maize hybrids grown in Serbia in the 2014-2017 period*
P202 **Rosa Ana Malvar** <rmalvar@mbg.csic.es>	*GWAS for resistance to mediterranean corn borer and agronomic traits in a MAGIC population of maize*
P203 **Torbert Rocheford** <torbert@purdue.edu>	*GWAS of inflorescence architecture in maize*
P204 **Gladys Cassab** <gladys@ibt.unam.mx>	*Hydrotropism: an important root trait for drought and heat avoidance in maize*

P205 **Yu Zhong** <zhongyu306@cau.edu.cn>
Identification of haploid immature embryos by their morphological difference in maize

P206 **Aimee J. Schulz** <aschulzj@iastate.edu>
Inbreeding depression in wild maize populations (Zea mays ssp. parviglumis) subject to habitat degradation in southwest Mexico

P207 **Guillaume Ramstein** <gr226@cornell.edu>
Incorporation of functional information into genomic prediction models in maize

P208 **Peter Muth** <p.muth@lmu.de>
Influence of arbuscular mycorrhiza on stress resilience in a European maize diversity panel

P209 **Valerie Craig** <craigv@uoguelph.ca>
Inheritance of short-season maize (Zea mays L.) senescence patterns

P210 **Mike White** <mrwhite4@wisc.edu>
Insights into heterotic patterns and allelic diversity of U.S. dent maize from expired plant variety protection inbreds

P211 **Heather Manching** <hcorn@udel.edu>
Investigating the genetics of selection response for flowering time in a multi-environment, parallel selection experiment.

P212 **Inoussa Sanane** <inoussa.sanane@u-psud.fr>
Maize resistance to parasitism : life-cycles synchronisation matters

P213 **Merritt Burch** <merritt.b.burch@sdstate.edu>
Mapping loci that modify the efficacy of Teosinte crossing barrier 1

P214 **Silvio Salvi** <silvio.salvi@unibo.it>
Mapping QTLs for Kernel Row Number and Fasciated Ear by SNP-Based Bulk Segregant Analysis in Maize

P215 **Florian Berger** <flo.berger@lmu.de>
MAZE - Accessing arbuscular mycorrhiza-mediated drought stress resistance in a maize diversity panel

P216 **Mark Holmes** <holme616@umn.edu>
Natural variation for starch composition and processing characteristics of maize

P217 **Laura Manerus** <lmanerus@uoguelph.ca>
Navigating the maize of short-season ancestry

P218 **Felix Seifert** <felix.seifert@cropseq.com>
Omics-based prediction of hybrid grain yield in maize

P219 **Calli Anibas** <canibas@wisc.edu>
Phenotypic and genetic dissection of cold tolerance in maize using controlled environments and in-field evaluations

P220 **Antoine Allier** <antoine.allier@inra.fr>
Phenotypic and genomic indicators of breeding program sustainability: application to a north European grain maize program

P221 **Thomas Altmann** <altmann@ipk-gatersleben.de>
Plant phenotyping reveals genetic and physiological factors of plant performance

P222 **Hyeyoung Lee** <leehye@missouri.edu>
Plant Transformation Services

P223 **Snezana Mladenovic Drinic** <msnezana@mrizp.rs>
Population structure and genetic diversity of different maize genotypes

P224 **Anna Giulini** <annapiamaria.giulini@crea.gov.it>
Post breeding

P225 **Domagoj Simic** <domagoj.simic@poljinos.hr>
QTL for photosynthetic and yield performance in IBM population under two different heat scenarios during flowering time

P226 **Adama Seye** <adama.seye@inra.fr>
QTL mapping and genomic predictions for silage quality traits in a multiparental hybrid design

P227 **Popi Septiani** <popi.septiani@santannapisa.it>
QTL mapping for fusarium seedling rot resistance in the recombinant inbred crosses derived from MAGIC maize population

P228 **Kathleen Miller** <kmiller46@wisc.edu>
QTLs affecting sweet corn carbohydrate content and eating quality in sugary1

P229 **Slavica Stankovic** <sstojkov@mrizp.rs> — *Relationships between resistances to Fusarium and Aspergillus ear rots and mycotoxins contamination in maize kernels*

P230 **Mihai Miclaus** <mihai.miclaus@icbcluj.ro> — *Romania's 3,000 inbred lines collection as a reservoir of genetic diversity and the use of its cytolines in linking phenotype to genotype*

P231 **Felix Frey** <ffrey@uni-bonn.de> — *Root phenotypic and transcriptomic variation in the European maize landrace Petkuser in response to cold*

P232 **Delphine SteinbachH** <delphine.steinbach@inra.fr> — *ThaliaDB, a tool for data management and genetic diversity data exploration*

P233 **Arnaud Desbiez-Piat** <arnaud.desbiez-piat@u-psud.fr> — *The dynamics of adaptive response under strong selection regime in small populations*

P234 **Bridget McFarland** <bamcfarland@wisc.edu> — *The effects of artificial selection on stability and GxE in the Iowa stiff stalk synthetic maize population*

P235 **Jason Wallace** <jason.wallace@uga.edu> — *The effects of host and environment on the maize microbiome*

P236 **Clement Buet** <clement.buet@biogemma.com> — *The phenotypic characterization of the BALANCE maize panel reveals high potential to discover genetic determinants involved in drought response*

P237 **Nicola Bacciu** <nicola.bacciu@keygene.com> — *The ProSpect of traditional and molecular breeding.*

P238 **Anne Zanetto** <anne.zanetto@inra.fr> — *The Zea French Biological Resource Centre: conservation and utilization of maize genetic resources in France*

P239 **Catherine Giauffret** <catherine.giauffret@inra.fr> — *Three chromosomal segments have a strong effect on photosynthesis and ability of maize plants to transition to autotrophy under chilling conditions*

P240 **Karin Ernst** <karin.ernst@hhu.de> — *Towards cloning of a major chilling tolerance QTL in maize*

P241 **Bernardo Ordas** <bordas@mbg.csic.es> — *Transcriptomic analysis of senescence in maize inbred lines with different rate of senescence*

P242 **Xianran Li** <lixr@iastate.edu> — *Turbocharging germplasm banks: genomic prediction goes into micro-world*

P243 **Elise Tourrette** <elise.tourrette@inra.fr> — *Unleashing genetic diversity by increasing meiotic recombination : an in silico benchmark*

P244 **Brian Rice** <brice6@illinois.edu> — *Utilizing GWAS Results to Preferentially Treat Genomic Markers in Prediction Model*

P245 **Tao Zhong** <zhongtaomvp@163.com> — *ZmAuxRP1, encoding an auxin-regulated protein, coordinates the balance between growth and defense in maize*

P246 **Jinge Tian** <tianjingelove@126.com> — *ZmCCT9 enhances maize adaptation to higher latitudes*

P247 **Yingjia Han** <hanyingjia325@163.com> — *ZmCOP II controls oil content in maize kernel*

Transposons & Epigenetics

P248 **Chunguang Du** <duc@montclair.edu> — *A single gene knock-out resource for maize: filling gaps in the genome with targeted Ds-GFP insertions*

P249 **Donald McCarty** <drm@ufl.edu> — *Accuracy of the UniformMu resource is improved 15% by mapping Mu insertions in its native W22 genome*

P250 **Michelle Stitzer** <mcstitzer@ucdavis.edu> — *Atypical transposable element copies predict functional consequence*

P251 **Yubin Li** <liyubin@caas.cn>
Buried treasures: the maize transposable elements Dotted and Mrh

P252 **Emily McCormic** <mccormic.11@osu.edu>
Characterization and mapping of the required to maintain repression10 locus affecting paramutation

P253 **Alex Brohammer** <broha006@umn.edu>
Characterization of polymorphic transposable element content between maize inbred lines

P254 **Clémentine Vitte** <clementine.vitte@inra.fr>
Cold induces transcriptional and methylation changes in the sensitive line B73

P255 **Cristian Forestan** <cristian.forestan@unipd.it>
Discovering the epigenetic memory of stress response in maize

P256 **Jian Chen** <jianchen@cau.edu.cn>
Distinct pattern of DNA methylation in different submucleosomal domains

P257 **Jaclyn Noshay** <nosha003@umn.edu>
Documenting the role of transposable elements in DNA methylation variation in maize

P258 **Maike Stam** <m.e.stam@uva.nl>
Identification and characterization of regulatory sequences in Zea mays

P259 **Caroline Marcon** <marcon@uni-bonn.de>
Identification of new maize (root) mutants by Mu-seq

P260 **Na Wang** <na.wang25@uga.edu>
Maize centromeres expand in the large genome background of Oaxaca and Zea. luxurians

P261 **Tong Li** <litong@cau.edu.cn>
Parent-of-origin dependent nucleosome organization and its role on the regulation of genetic imprinting in maize endosperm

P262 **Susanne Edelmann** <susanne.edelmann@uni-hamburg.de>
Reduction of DNA methylation during early embryogenesis enhances growth heterosis of maize plants

P263 **Jay Hollick** <hollick.3@osu.edu>
RNA polymerase IV contributes to hybrid vigor

P264 **Benjamin Berube** <bberube@cshl.edu>
Single-pollen sequencing for the study of novel meiotic phenotypes

P265 **Surinder Chopra** <sic3@psu.edu>
The maize Ufo1 mutant results from ectopic over expression of an endosperm specific gene

P266 **Thomas Brutnell** <tbrutnell@danforthcenter.org>
The transposon landscape of the inbred W22

P267 **William Ricci** <william.ricci@uga.edu>
Using chromatin features to identify and understand intergenic transcriptional regulatory elements in maize

P268 **Allison McClish** <mcclish.23@osu.edu>
Using GRO-Seq as a tool to understand transcriptional regulation in maize

Plenary Talk Abstracts

Plenary 1 **Friday, March 23 11:15 AM**

Epigenetic variation in Arabidopsis

(presented by Magnus Nordborg <magnus.nordborg@gmi.oeaw.ac.at>)
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Epigenetics continues to fascinate, especially the notion that it blurs the line between "nature and nurture" and could make Lamarckian adaptation via the inheritance of acquired characteristics possible. That this is in principle possible is clear: in the model plant Arabidopsis thaliana (thale cress), experimentally induced DNA methylation variation can be inherited and affect important traits. Less clear is whether this matters in nature. Recent studies of A. thaliana have revealed a pattern of correlation between levels of methylation and climate variables that strongly suggests that methylation is important in adaptation. However, somewhat paradoxically, the experiments also showed that much of the variation for this epigenetic trait appears to have a genetic rather than an epigenetic basis. This suggest that epigenetics may indeed be important for adaptation, but as part of a genetic mechanism that is currently not understood. Genome-wide association studies revealed a striking genetic architecture of methylation variation, involving major-effect polymorphisms in many genes involved in silencing, and we are currently utilizing this to determine whether the global pattern of methylation variation has a genetic or an epigenetic cause, and to use this information to elucidate the ultimate cause of the global pattern of variation: natural selection.

Drought tolerance: which mechanisms, traits and alleles for which drought scenarios?

(presented by Francois Tardieu <francois.tardieu@inra.fr>)
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Plants are subjected every day to rapid variation of evaporative demand and soil water availability, resulting in rapid changes in stomatal conductance, expansive growth and metabolism over minutes. Because yield involves several months, the connection between physiological mechanisms and response of yield to drought scenarios faces a massive problem of time scales. Furthermore, yield results from optimization between traits and alleles that lead to either minimize the risk of crop failure or to increase crop production. Evolution has tended to favour conservative processes (short crop cycle, low transpiration and leaf area, large root systems) which are favourable under severe stresses, whereas yield in milder water deficits is associated with the opposite traits. Hence, one aims at identifying which traits and alleles are favourable in which drought scenarios, rather than at a generic 'drought tolerance'. We deal with these methodological difficulties by combining phenomics, modelling, genetic analysis and genomic prediction. A first strategy explores the genetic variability of key processes, which are translated into parameters of a crop model. This requires detailed analyses in phenotyping platforms with a capacity of thousands of plants, with the relevant time scales. These parameters are analysed by GWAS and simulated via genomic prediction. The model can then simulate yield in hundreds of fields for hundreds of genotypes, from genetic parameters of each genotype and environmental conditions in each field. A second strategy directly explores the responses of yield to environmental conditions in contrasting environmental scenarios, e.g. in 40 fields. This results in a mixed model whose parameters are analysed genetically and can be estimated by genomic prediction, thereby allowing one to predict yields in new genotypes and fields. As a whole, the combination of field and platform data allows identification of combination of traits and alleles associated with tolerance in specific scenarios of heat and drought.

Funding acknowledgement: INRA, ANR, UE

Light control of seed germination: the unusual role of usual suspects

(presented by: Ortun Mittelsten Scheid <ortrun.mittelstenscheid@gmi.oeaw.ac.at>)
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Seed germination is a sensitive period in a plant's life cycle, and initiation of the process must be concerted with many internal and external factors. Light is an important parameter, extensively documented for the model plants Arabidopsis thaliana and Lactuca sativa, in which light exposure of seeds is required to trigger a well-investigated signalling cascade resulting in hormonal control of germination. In contrast, seeds of other plants germinate equally well in light or darkness, and seeds of some species do not germinate at all as long as they are exposed to light. The mechanism of an inhibiting role of light on seed germination is not understood.

The talk will present physiological and molecular data generated in experiments with Aethionema arabicum, a member of the Brassicaceae and related with Arabidopsis. While gibberellins and abscisic acid are involved in the control of germination in both species, the light-induced changes of the ratio between the two hormones are antipodal between Aethionema and Arabidopsis. Genomic information, natural variation, and differential expression of regulatory genes suggest Aethionema as a suitable model plant to investigate the molecular mechanism of germination inhibition by light. The data indicate that similar modular components have been assembled by evolution in different ways to produce divergent pathways.

A bad genetic history of maize.

(presented by: Jeffrey Ross-Ibarra <rossibarra@ucdavis.edu>)
Full Author List: Ross-Ibarra, Jeffrey[1]
[1] Department of Plant Sciences, University of California - Davis

Deleterious alleles have played an important role in the evolution of maize and teosinte. Although they vary in their strength and effect across populations or environments, such mutations have played a role in local adaptation in teosinte, the accumulation of load during domestication and dispersal of maize, local adaptation of maize landraces, and ultimately in hybrid vigor for agronomic traits in breeding programs.

Germline reprogramming, epigenetic inheritance and small RNA: how to avoid Bad Karma.

(presenter: Rob Martienssen <martiens@cshl.edu>)
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The germlines of animals and plants undergo reprogramming to reset epigenetic marks acquired during development, and to restore pluripotency to the zygote. When reprogramming fails, epigenetic inheritance results. Epigenetic inheritance is far more common in plants than in mammals, as demonstrated by the early work of B. McClintock and R.A. Brink on transposon cycling and paramutation in maize. We have found that in Arabidopsis pollen, genome reprogramming in sperm cells results in the loss of RNA dependent DNA methylation, and the accumulation of a new class of "epigenetically activated" small RNA (easiRNA). A highly conserved microRNA, miR845, targets the primer-binding site (PBS) of LTR-retrotransposons and triggers the accumulation of 21 to 22-nucleotide easiRNA in a dose dependent fashion. These easiRNAs mediate hybridization barriers between diploid seed parents and tetraploid pollen parents ("the triploid block"). Natural variation for miR845 among Arabidopsis accessions may account for "endosperm balance" to allow formation of triploid seeds. After fertilization, DNA methylation is restored in the embryo, guided by small RNA. When reprogramming fails, for example in Arabidopsis cell culture, easiRNA also accumulate and RdDM is lost. In a real world example, accumulation of easiRNA and the loss of RdDM in oil palm tissue culture results in the epigenetic inheritance of the "mantled" somaclonal abnormality. Mantled is caused by aberrant splicing of the Karma retrotransposon, found in the intron of the Deficiens gene. A simple DNA methylation test can predict mantled clones, providing a robust screen to avoid planting infertile material in environmentally sensitive tropical plantations.

Short Talk Abstracts

SESSION 1 – THE GENES THAT MAKE MAIZE
Chair: Andrea Gallavotti Thursday, March 22. 7:30 PM – 9:25 PM

T1

How to make maize seeds that look "not like dad": insights in double fertilization and prospects for novel breeding tools.

(submitted by Thomas Widiez <thomas.widiez@ens-lyon.fr>)
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Mixing male and female genetic information during sexual reproduction is considered as key to the evolutionary success of higher eukaryotes and is the basis of plant breeding. Sexual reproduction in flowering plants involves double fertilization, characterized by two separate fusion events between the male and female gametes. A maize line first reported in the 60s deviates from this classic pattern. Crosses using pollen from this so-called haploid inducer line, trigger the development of the egg cell into a haploid embryo with only the maternal genome, a process known as *in vivo* gynogenesis. Derivatives of this maize haploid inducer line have become the preferred tool of numerous maize breeding companies, because it can produce perfectly homozygous plants in only 2 generations instead of 5 to 8 in classical breeding schemes. Our recent results (Gilles et al., EMBO J), together with two other simultaneous independent studies (Kelliher et al., Nature; and Liu et al., Molecular Plant), identified the major causal gene responsible for gynogenesis in maize. Our map based cloning restricted the QTL to a zone containing a single gene coding for a patatin-like phospholipase A, which was named *NOT LIKE DAD* (*NLD*) because haploid embryos do not have paternal contribution. In all surveyed haploid inducer lines *NLD* carries a 4 pb insertion leading to a predicted truncated protein. This frameshift mutation is responsible for haploid induction as complementation with wildtype *NLD* abolishes the haploid induction capacity. Translational NLD::citrine fusion protein likely localizes to the sperm cell plasma membrane. In Arabidopsis roots, the truncated protein is no longer localized to the plasma membrane, contrary to the wildtype NLD protein. In conclusion, an intact sperm-specific phospholipase is required for successful sexual reproduction and its targeted disruption may allow establishing powerful haploid breeding tools in numerous crops.

Gene / Gene Models described: *not like dad*; GRMZM2G471240 or Zm00001d029412

T2

Generating clonal progeny in maize

(submitted by Nina Chumak <nina.chumak@botinst.uzh.ch>)
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Apomixis is asexual reproduction through seed. The production of seeds though apomixis, which generates plants that are genetically identical to the mother plant, has considerable agricultural potential to maintain desired complex genotypes, e.g. those of F1 hybrids, over many generations.
Gametophytic apomixis deviates from sexual development in three major steps: (1) meiosis is circumvented or aborted, leading to the formation of unreduced, unrecombined embryo sacs (apomeiosis); (2) embryogenesis initiates without fertilization of the unreduced egg cell (parthenogenesis); and (3) developmental adaptations enable the formation of functional endosperm. The aim of our research is to identify mutations that mimic the major components of apomixis, and to combine them to engineer apomictically-reproducing maize plants.
In a genetic screen we identified the non-reduction in female 4 (*nrf4*) mutant, which mimics the first step of apomixis: apomeiosis. Homozygous *nrf4* plants produce up to 95% unreduced embryo sacs. Using SAIFF-by sequencing technology, the mutation was mapped to GRMZM2G148133 on the long arm of chromosome 7, and the identity of *Nrf4* was confirmed by two additional mutant alleles. To identify whether *nrf4* leads to first or second division restitution (FDR vs SDR), we analyzed maintenance of heterozygosity in the progeny of *nrf4* mutant plants in comparison to mother plants using a SNP array that enabled the analysis of 10-20 SNPs on each chromosome. The effect of the *nrf4* mutation turned out to be more complex than expected and leads to both FDR and SDR. Nonetheless, depending on the genetic background of the mother plant, up to 11% of the unreduced female gametes were genetically identical to the mother. Indeed, pollination of *nrf4* plants by a tetraploid haploid inducer resulted in some clonal individuals. To our knowledge this is the first evidence that production of clonal individuals through seed is possible in maize.

Gene / Gene Models described: *Nrf4*; GRMZM2G148133
Funding acknowledgement: DuPont/Pioneer, SNF

T3

Maize *Carbohydrate partitioning defective33* functions in sucrose export from leaves

(submitted by Thu Tran <tmtqk3@mail.missouri.edu>)
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To sustain plant growth, development, and ultimately crop yield, sucrose must be transported from its site of synthesis in leaves to distant parts of the plant, such as seeds or roots. Yet we know little about the genes controlling carbohydrate distribution in plants. Here we discuss our exciting discovery of a gene impacting sucrose export from maize leaves.

carbohydrate partitioning defective33 (cpd33) is a recessive mutant, which accumulates excess starch and soluble sugars in the mature leaves. Additionally, *cpd33* mutants exhibit chlorosis in the leaf blades, greatly diminished plant growth, and reduced fertility. Furthermore, application of radioactively labeled F18-sucrose to *cpd33* mutant and wild-type leaves showed that sucrose export was greatly decreased in *cpd33* mutant leaves compared with wild type. The *Cpd33* gene has been cloned by genetic fine-mapping and whole genome sequencing experiments, and its identity confirmed through characterizing multiple mutant alleles. The *Cpd33* gene encodes an evolutionarily conserved plant-specific protein predicted to contain multiple transmembrane domains. In tobacco leaves, a CPD33-yellow fluorescent protein translational fusion protein is associated with the plasma membrane; however, the signal appears discontinuously along the membrane, suggesting that CPD33 is localized at plasmodesmata.
Based on these results, we propose that CPD33 functions to control sucrose export from leaves through regulating cell-to-cell transport through plasmodesmata. Our ongoing work utilizes molecular approaches in combination with imaging techniques to test models of CPD33 function. This research reveals a new gene involved in sucrose export and deepens our understanding of the control of carbohydrate partitioning.

Funding acknowledgement: National Science Foundation (NSF)

T4

NARROWSHEATH1 controls cell cycle dynamics to promote mediolateral outgrowth of leaf primordia

(submitted by Phillip Conklin <pac257@cornell.edu>)
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The mechanisms whereby lateral organ initial cells are organized from the peripheral zone of the shoot apical meristem (SAM) are poorly understood in grasses. The maize WUSCHEL-LIKE HOMEOBOX3 (WOX3) gene NARROWSHEATH1 (NS1) is expressed at the marginal boundary of leaf initial cells in the SAM and in young leaf primordia, where it mediates mediolateral outgrowth. This research teases apart conserved mechanisms of lateral organ initial cell recruitment versus stem cell organization in the central zone of shoot meristems and the quiescent center of root meristems. Although several studies have elucidated the network in the stem cell organizing center in eudicots, these employed overexpression of the stem cell organizing protein WUS1. We utilize domain-specific analysis of NS1 bound and modulated targets within the lateral organ initial cells, in maize. In so doing, we investigate whether organization of lateral organ initials and stem cells in the root (via WOX5) and shoot apical meristems (via WUS and NS1/WOX3) have the same general functions in distinct developmental domains or in contrast, if they perform distinct, domain-specific functions. To elucidate the genetic regulation of lateral organ initials, we compare ChIP-seq targets bound by NS1 and RNA-seq transcripts modulated in laser micro-dissected early leaf margins. These experiments, combined with microscopic analyses of cell division dynamics, suggest that NARROWSHEATH1 controls mediolateral outgrowth by direct regulation of cell cycle genes and growth regulators at the margins of the developing leaf primordia.

Gene / Gene Models described: *NS1*; GRMZM2G069028
Funding acknowledgement: National Science Foundation (NSF)

T5

Interrogating metabolic and transcriptomic networks to explain genetic, developmental and environmental variation in the cuticular lipid landscape on maize silks

(submitted by Keting Chen <kchen@iastate.edu>)
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The plant cuticle is infused with and coated by non-polar and amphipathic lipids that form a hydrophobic layer that is protective against environmental stresses. These extracellular surface lipids (SLs) are comprised primarily of long-chain saturated and unsaturated fatty acids, aldehydes, and hydrocarbons, which are metabolically linked by enzymatic reactions as the hypothesized precursors, intermediates, and end products in hydrocarbon biosynthesis. To investigate this biosynthetic pathway, we employed a systems approach to query the metabolomes and transcriptomes of silks from four genotypes (B73, Mo17 and their reciprocal hybrids) across a spatio-temporal gradient that captures acropetal silk development and the environmental transition as silks emerge from the husks.

Supervised and un-supervised network analyses were pursued to address key questions: 1) Which metabolites explain the dynamic variations in SL composition? 2) Which enzymatic processes lead to variation in these metabolites? and 3) What genes explain the differential metabolome compositions? Our results show that silk SL composition is dynamic and significantly impacted by encasement status, genotype, and development. Discriminant analysis revealed that differential utilization of fatty acid precursors likely contributes to the observed variation in hydrocarbon composition among genotypes. Product-precursor ratio investigations showed that hydrocarbon abundances are elevated relative to their associated fatty acid precursors at longer chain-lengths, suggesting increased recruitment of longer-chain fatty acid precursors into the biosynthetic pathway. Metabolome-transcriptome associations impacting hydrocarbon production under varied conditions were identified from a partial least squares regression model built from a set of informative metabolites. Preliminary analysis identified candidate genes associated with genotype-based variation in the metabolic network, including 3-ketoacyl-CoA synthases involved in generating fatty acid precursors, and acyl desaturases involved in production of unsaturated SLs. Analyses are being conducted to interrogate the transcriptome in the context of product-precursor, product-intermediate and intermediate-precursor relationships to identify candidate genes associated with specific biochemical reactions in the network.

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

T6

How polygenic adaptation during domestication shaped the genetic architecture of maize

(submitted by Markus Stetter <mstetter@ucdavis.edu>)
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The domestication of maize has radically changed its morphology and physiology. A number of genes controlling domestication traits have been identified over the last decades, however, these genes only explain a fraction of the variation observed for most traits. Genome wide association mapping and genomic prediction studies show that many traits are controlled by a large number of loci with a variety of effect sizes. To better understand the genetic architecture of complex traits during domestication, we explicitly simulated quantitative trait evolution under selection and demographic models specific to maize history. To explore different traits, we varied the effect size distribution of incoming mutations, the strength of stabilizing selection and the genomic background, and used machine learning to identify the relative importance of different parameters. We show that traits exhibiting the greatest phenotypic change and strongest stabilizing selection in teosinte rely on new beneficial mutations to adapt. Mutations with positive effects sweep to fixation, resulting in a large number of both soft and hard selective sweeps. Traits under weaker stabilizing selection and exhibiting less phenotypic change adapt via a subtle shift at many loci resulting in only few selective sweeps, mostly from genotypic variation that was already present in teosinte. These subtle shifts in allele frequencies maintain larger genetic variance, but also large effect negative alleles that are deleterious for further breeding. We show how the effect size of incoming mutations, the strength of stabilizing selection, the genomic background and population demography influence adaptation, genetic architecture and the interplay between polygenic adaptation and selective sweeps. Our results illustrate that polygenic adaptation and selective sweeps are not mutually exclusive but can coexist, depending on various parameters. We further elucidate the genomic patterns observed in modern maize, and provide the opportunity to identify new targets for genetic improvement.

Funding acknowledgement: National Science Foundation (NSF), Deutsche Forschungsgemeinschaft (DFG)

T7

Twenty years of divergent selection for flowering time from maize inbred lines

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Two independent Divergent Selection Experiments (DSEs) for flowering time in maize have been conducted under agronomical conditions in Plateau de Saclay for more than twenty generations. The two initial populations consisted in two seed lots, each from a single inbred line. At each generation, we selected and selfed early and late flowering genotypes. By selecting from such a narrow genetic basis, we expect to have enriched populations for (epi)genetic differences controlling flowering time, while preserving the original characteristics of the initial inbreds. We used this material to investigate (i) dynamics of the response to selection by comparing genotypes among generations (ii) genotype-phenotype map by comparing Early and Late genotypes within DSEs; (iii) patterns of convergence by comparing Early (Late) genotypes between DSEs.

Altogether, we observed a significant response to selection in both directions (0.5 days/year on average), with strikingly similar patterns for both DSEs. A revised version of the animal model that explicitly accounts for the role of new mutations showed that both residual heterozygosity and new mutations contributed to the selection response.

Early and Late progenitors from generations G13 and G18 were used to perform in-depth characterization of gene expression, as well as plants growth and development. At the phenotypic level, differences between Early and Late genotypes concerned primarily the timing of developmental transitions, while organs' growth rates and phyllochrons were much less variable. RNA-Seq analyses on shoot apical meristems revealed 2,451 genes that were differentially expressed between Early and Late genotypes during floral transition (candidate genes), with more common candidates between the two DSEs than expected by chance (convergence). Finally, using *Lepidoptera* stem borers as a model, we analysed how plant phenology shifts interfered with pest life-cycle. We found higher prevalence in one Early population, synchronized with the arrival of the second generation of adult insects.

Funding acknowledgement: INRA, Labex BASC

T8

Prehistoric selection for temperate climates has far-reaching implications for global germplasm

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Researchers have long recognized that flowering time in maize is highly correlated with other agronomically important traits, with implications for trait introgression and breeding. We show that this association between flowering and modern population structure is at least 2,000 years old and dates to the historic temperate adaptation process as maize moved north out of Mexico to the temperate southwest United States. We then test the impact of this process on agronomically important global germplasm: the Ames population and US-NAM (American), the EU-NAM Flint and Dent panels (European), and CN-NAM (Chinese). We use a random forest classifier to test the prediction accuracy of population differentiation between American temperate and tropical germplasm on uncorrected GWAS results from these families. Because these populations derive from geographically restricted germplasm with variance for flowering, accurate prediction implies a shared selection history. Prediction accuracies (AUC) averaged across chromosomes are 0.89 for Ames, 0.85 for US-NAM, 0.67 for EU-NAM Flint, 0.59 for EU-NAM Dent and 0.47 for CN-NAM, suggesting that the North American temperate adaptation process was important for European temperate adaptation but that the temperate Chinese germplasm derives from an unrelated selection process.

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T9

Distinct Genetic Architectures for Phenotype Means and Plasticities in *Zea mays*

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Phenotypic plasticity describes the phenotypic variation of a trait when a genotype is exposed to different environments. Understanding the genetic control of phenotypic plasticity in crops such as maize is of paramount importance for maintaining and increasing yields in a world experiencing climate change. Here, we report the results of genome-wide association analyses of multiple phenotypes and two measures of phenotypic plasticity in the maize nested association mapping (US-NAM) population grown in multiple environments and genotyped with ~2.5 million single nucleotide polymorphisms (SNPs). We show that across all traits the candidate genes for mean phenotype values and plasticity measures form structurally and functionally distinct groups. Such independent genetic control suggests that breeders will be able to select semi-independently for mean phenotype values and plasticity, thereby generating varieties with both high mean phenotype values and levels of plasticity that are appropriate for the target performance environments.

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T10

The conserved and unique genetic architecture of kernel size and weight in maize and rice

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Maize (*Zea mays*) is a major staple crop. Maize kernel size and weight are important contributors to its yield. Here, we measured kernel length, kernel width, kernel thickness, hundred kernel weight and kernel test weight in 10 recombinant inbred line populations and dissected their genetic architecture using three statistical models. In total, 729 quantitative trait loci (QTLs) were identified, many of which were identified in all three models, including 22 major QTLs that each can explain more than 10% of phenotypic variation. To provide candidate genes for these QTLs, we identified 30 maize genes that are orthologs of 18 rice (*Oryza sative*) genes reported to affect rice seed size or weight. Interestingly, 24 of these 30 genes are located in the identified QTLs or within 1 Mb region of the significant single nucleotide polymorphisms (SNPs). We further confirmed the effect of five genes on maize kernel size/weight in an independent association mapping panel with 540 lines by candidate gene association analysis. Lastly, we mapped a major QTL (*qHKW1*) for hundred kernel weight into ~650Kb region harboring 9 genes. Among these 9 genes, only 2 were reported to express during maize kernel development and one of them (*qHKW1-9*) was annotated to involve in plant development. Transgenic lines that overexpress *qHKW1-9* have significant increase in hundred kernel weight compared to the negative transgenic lines. Our findings shed light on the genetic basis of kernel size and weight in maize and provided evidence for a conserved and unique genetic architecture of kernel traits in maize compared with rice.

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T11

Genome-wide nucleotide divergence and UV induced mutations following maize domestication

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Maize domestication provides an ideal system to understand evolution. The history of maize domestication has been well documented with tremendous morphological changes, adaptation to diverse environments, and major demographic shifts. Meanwhile, maize genome also went through profound changes following domestication bottleneck, *i.e.*, domesticated maize have significantly reduced genetic diversity and increased mutational load. More recently, a striking pattern, maize building their genomes with more nucleotide A and T than their wild progenitor teosinte, was discovered. This genome divergence pattern was consistently observed in multiple other species.

Here, by focusing on the base-composition value summarized from polymorphic sites, we provide novel insights on maize genome evolution at different regions of the genome: genic versus nongenic, pericentromeric versus nonpericentromeric, and methylated versus unmethylated. We show that the divergence in base-composition value between maize and teosinte is larger in nongenic than genic part of the genome. Interestingly, the divergence is significantly enlarged in pericentromeric regions. Moreover, motif frequency and sequence context analyses showed the motifs (PyCG) related to solar-UV signature have higher frequencies in nongenic and pericentromeric regions, particularly when they are methylated, implicating the role of DNA methylation in promoting solar-UV induced mutations. We also discovered the enrichment of mutations related to solar-UV signature in maize, which indicates the varied mutation rate across populations. In addition, a set of genes including *ATR* and *RPA1* involved in UV damage repair pathways were identified to be associated with the genome divergence between maize and teosinte.

Together, these findings demonstrate that solar-UV radiation and differential mutation repair play a critical role in the genome divergence between maize and teosinte. Our integrated analysis provides the first example to establish the important links among UV radiation, mutation, DNA repair, methylation, and genome evolution.

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T12

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

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We created a new high-coverage, robust, and reproducible functional annotation of maize protein coding genes based on Gene Ontology (GO) term assignments. Whereas the existing Phytozome and Gramene maize GO annotation sets only cover 41% and 56% of maize protein coding genes, respectively, this study provides annotations for 100% of the genes. We also compared the quality of our newly-derived annotations with the existing Gramene and Phytozome functional annotation sets by comparing all three to a manually annotated gold standard set of 1,619 genes where annotations were primarily inferred from direct assay or mutant phenotype. Evaluations based on the gold standard indicate that our new annotation set is measurably more accurate than those from Phytozome and Gramene. To derive this new high-coverage, high-confidence annotation set we used sequence similarity and protein-domain-presence methods as well as mixed-method pipelines that developed for the Critical Assessment of Function Annotation (CAFA) challenge. Our project to improve maize annotations is called maize-GAMER (GO Annotation Method, Evaluation, and Review) and the newly-derived annotations are accessible via MaizeGDB (http://download.maizegdb.org/maize-GAMER) and CyVerse (B73 RefGen_v3 5b+ at doi.org/10.7946/P2S62P and B73 RefGen_v4 Zm00001d.2 at doi.org/10.7946/P2M925).

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T13

Using DAP-seq to map genome-wide ARF transcription factor binding events in maize

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AUXIN RESPONSE FACTORS (ARFs) are a family of plant-specific transcription factors (TFs) that occupy a pivotal position in the signal transduction pathway of the plant hormone auxin. Due to their ability to directly bind DNA and to interact with components of the auxin receptor, ARFs mediate the large transcriptional changes and diverse developmental responses regulated by auxin. However, our understanding of the functional differences among ARF members is severely limited by a lack of genome-wide binding data for the numerous family members that are present in higher plant species. Using DAP-seq, a genomic DNA-TF binding assay, we obtained comprehensive cis-regulatory maps for fourteen maize ARFs from the two evolutionarily conserved class A 'activator' and class B 'repressor' clades. We identified 124,530 distinct binding sites, generating the largest dataset of ARF targets identified in any plant species to date. We observed minimal genome-wide binding site diversity for ARFs of the same class, but found substantial differences in motif sequence, spacing, site preference, and association with auxin induced genes among class A and class B ARFs. Despite these differences, many binding sites and target loci were occupied by ARFs from both classes, suggesting transcriptional coordination for a large number of genes related to maize development, domestication and fitness, including putative regulatory targets that correspond to known QTL regions.

Gene / Gene Models described:
ARF34,ARF16,ARF27,ARF4,ARF29,ARF35,ARF18,ARF25,ARF14,ARF7,ARF10,ARF36,ARF13,ARF39; GRMZM2G081158,GRMZM2G028980,GRMZM2G160005,GRMZM2G034840,GRMZM2G086949,GRMZM2G317900,GRMZM2G035405,GRMZM2G116557,GRMZM2G137413,GRMZM2G475263,GRMZM2G338259,GRMZM2G702026,GRMZM2G378580,GRMZM2G017187
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T14

Long-range Interactions Provide a Topological Basis for Genetic Regulation of Complex Traits in Maize

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Higher-order chromosomal organization for transcription regulation is poorly understood in plants. We performed genome-wide chromatin interaction analysis by paired-end-tag sequencing (ChIA-PET), and identified more than 20,000 long-range enhancer- and promoter-centered chromatin interaction associated with H3K4me3 and H3K27ac in maize immature ear and shoot. The P-P, P-E, and E-E interactions accounted for 65%, 35%, and 5% chromatin interaction between enhancer and promoter, respectively. Genes which promoters interacted with each other preferred to be co-expressed. The expression levels of genes with interacting enhancers are higher than that of genes without interacting enhancers. In addition, compared to genes with intergenic interacting enhancers, genes with enhancers in gene bodies are prone to be constitutive genes. Comparison of chromatin interactions between tissues revealed that tissue-specific chromatin organization is associated with the expression of tissue-specific genes. Moreover, the P-E interactions map also provided a topological basis for the transcription regulation of genes associated with complex agronomic traits.

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T15

The RNAseq Time Machine: Species-specific shifts in developmental timing and trajectory underlie morphological differences in maize tassel and sorghum panicle architecture

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The maize genetics community has identified key regulators of tassel architecture through the study of mutants and their interactors. By dissecting mutant inflorescence phenotypes, researchers have characterized a progression of tassel developmental stages. Yet our understanding of the global dynamics of gene expression and the developmental trajectory of genes outside of classic master regulators is still fragmented. On the other hand, *Sorghum bicolor* (L. Moench) is a close maize relative that creates a complex inflorescence of perfect flowers, or panicle, for which we have little developmental genetic information.

We took advantage of high-throughput sequencing, dynamic gene expression analysis techniques, and the rich history of maize genetics to stage and compare maize tassel and sorghum inflorescence development, independent of morphological features. By conducting tandem RNAseq and image processing on more than 40 individual maize tassel and sorghum panicle primordia across 40 days of development, we generated dense gene expression matrices that closely track the dynamics and trajectory of inflorescence development in both species. Using a core set of 1,500 developmentally-responsive transcripts, expression profile clusters identified about 5 developmental stages for both species. These stages strongly correlate with our collected inflorescence primordia morphological measurements. Dynamic gene expression patterns detected putative stage-specific molecular markers for each species and facilitated the comparison of sorghum and maize panicle development. By constructing gene co-expression networks for maize tassels and sorghum panicles, we generated large gene expression modules that correlate with developmental trajectory as well as morphological measurements. Expression modules were enriched for biological processes such as stem cell regulation and floral development and implicated previously unknown genes alongside familiar master regulators. Comparing stage-specific molecular markers, co-expression modules, and maize/sorghum syntenic orthologues of known inflorescence genes, we observed species-specific shifts in developmental trajectory and timing that may underlie morphological distinctions between these two essential food crops.

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T16

Enhancing phosphorus efficiency in maize and sorghum

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Phosphorus (P) is an essential nutrient to plants and is acquired as inorganic phosphate from the rhizosphere solution. P is one of the least available nutrients particularly in highly weathered, tropical soils, limiting substantially plant growth. Our work aimed to study root traits involved with P acquisition efficiency and to identify and validate maize and sorghum homologs to Phosphorus Starvation Tolerance 1 (PSTOL1), a gene responsible for enhanced early root growth, P uptake and grain yield in rice. Association mapping was undertaken in two sorghum association panels phenotyped for P uptake, root system morphology and architecture in hydroponics and grain yield and biomass accumulation under low-P conditions. SbPSTOL1 alleles reducing root diameter were associated with enhanced P uptake under low P in hydroponics, whereas other alleles increasing root surface area also increase grain yield in low-P soil. SbPSTOL1 genes colocalized with QTLs for traits underlying root morphology and dry weight accumulation under low P-soil. For maize, two multiple interval models were used to map QTLs related to root traits, biomass accumulation and P content in a maize RIL population cultivated in nutrient solution. Multiple interval mapping models for single and multiple traits were combined and revealed 13 genomic regions significantly associated with the target traits in a complementary way. Some of these quantitative trait loci (QTLs) were coincident with QTLs for root morphology traits and grain yield previously mapped, whereas others harbored ZmPSTOL1 candidate genes. OsPstol1 and its maize and sorghum homologs were cloned in the pMCG1005 vector and tobacco Petit havana plants were genetically transformed via Agrobacterium tumefaciens. Several events presented one copy of the transgene and those that also showed high transgene expression were selected for phenotypic evaluation under low P conditions. The transgenic plants, T1 and T2 generations, were grown for ~60 days in ½ MS medium with low P under controlled conditions. When compared with the control, plants transformed with pMCG1005 (empty vector) the PSTOL1 transgenic plants presented higher vegetative growth and root surface area under low P. Our results indicated that PSTOL1 homologs have a similar role as osPSTOL1 gene in rice plants and have potential to enhance P acquisition and yield in different species.

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T17

Regulation of aquaporin expression in maize: proximal and distal eQTLs

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Plasma membrane intrinsic proteins (PIPs) are aquaporins that facilitate the passive movement of water through biological membranes. These channels are involved in numerous physiological and cellular processes such as hydraulic conductivity, transpiration, photosynthesis and osmoregulation in plants. In a context of climatic changes, they could turn out to be key targets for selection of crop varieties optimized for water use efficiency.

Whereas *PIP* mRNA levels in maize tissues vary according to the developmental stages and under varying environmental conditions, the molecular actors and mechanisms regulating the transcription of these genes remain largely unknown. Relying on the genetic material (254 maize genotypes and ~1000 K SNPs collection) and expertise of the European DROPS (DROught tolerant yielding PlantS) project, we performed GWAS (genome-wide association study) analyses in order to identify promising eQTLs (expression quantitative trait loci) associated with variation in the expression of five *PIP* genes in the mature and elongation zones of the leaf. In most cases, highly significant eQTLs were identified close to the *PIP* genes themselves suggesting the large impact of *cis*-regulation, as well as numerous promising distal eQTLs that appear to be mainly zone-specific. Conditional GWAS, avoiding the *cis*-eQTLs effects, allowed the identification of even more proximal and distal eQTLs, highlighting the complex regulation of the PIP gene expression. Deeper analyses and functional validation of several eQTLs are currently ongoing.

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T18

Innovative and high-throughput field phenotyping method provides leaf traits for breeding of drought tolerance - From Leaf Area Index dynamics to its physiological components

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Since last decade genotyping advances, reliable, cheap, and high-throughput phenotyping methods has become critical to further dissect the genetics of quantitative traits. In maize breeding, substantial efforts are made to improve drought tolerance. Leaf development and senescence are pivotal in some of the major physiological strategies for drought tolerance. However, with classical phenotyping methods, monitoring leaf area in the field across maize whole lifecycle can be laborious, or even unfeasible for a large panel. We propose an innovative and high-throughput method to phenotype maize Leaf Area Index (LAI) dynamics and its components from emergence to death. Our approach is based on a simple LAI model, a few ground measurements and UAV remote sensing. For 15 contrasted genotypes of "BALANCE", a panel derived from a MAGIC population, we measured LAI model input parameters (phyllochron, final leaf number, biggest leaf area, and a senescence factor) in Well-Watered and Water-Deficient conditions. We then simulated LAI dynamics for these genotypes. All the 380 genotypes of the panel were measured on 9 dates across the lifecycle, with multispectral sensor mounted on a UAV, in both conditions. We built empirical relationships between multispectral measurements and simulated LAI of the contrasted genotypes and applied them to the entire panel. Based on this dataset we were able to inverse the LAI model and retrieve input parameters leading to these dynamics. Analyses showed that our 15 genotypes training dataset depicts fairly well the panel diversity when building the empirical relationships. They also indicated that multispectral data carry enough information to estimate LAI with good precision. Finally, LAI model inversion led to contrasted results: the senescence factor was retrieved with the higher accuracy, followed by biggest leaf area, phyllochron and final leaf number. We will discuss these results and draw the interesting prospects for breeding offered by this method.

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T19

Characterization of biosynthetic pathways and regulatory elements for the production of the volatile homoterpenes DMNT and TMTT in *Zea mays*

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Plant volatiles have multiple defense functions in the defense of maize against herbivores, fungi, and bacteria. In addition, these volatiles also have been implicated in signaling within the plant and towards other organisms. Elucidating the function of individual plant volatiles will require considerably more knowledge of their biosynthesis and regulation in response to external stimuli. Exploiting the variation of herbivore-induced volatile blends among 26 maize (*Zea mays*) inbred lines, we conducted a nested association mapping (NAM) and genome-wide association study (GWAS) to identify a set of quantitative trait loci (QTLs) for investigating the pathways of volatile terpene production. The most significant identified QTL affected the emission of (*E*)-nerolidol, linalool, and the two homoterpenes, 3,8-dimethyl-1,4,7-nonatriene (DMNT), and (*E,E*)-4,8,12-trimethyltrideca-1,3,7,11-tetraene (TMTT). GWAS associated this QTL with a single nucleotide polymorphism in the promoter of terpene synthase *tps2*. Biochemical characterization of TPS2 verified that this plastid-localized enzyme forms linalool, (*E*)-nerolidol, and (*E,E*)-geranyllinalool. The subsequent conversion of (E)-nerolidol into DMNT maps to a P450 monooxygenase, CYP92C5, which is capable of converting nerolidol into DMNT by oxidative degradation. A QTL influencing TMTT accumulation corresponds to a similar monooxygenase, CYP92C6, which is specific for the conversion of (*E,E*)-geranyllinalool to TMTT. The DMNT biosynthetic pathway and both monooxygenases are distinct from those previously characterized for DMNT and TMTT synthesis in Arabidopsis, suggesting independent evolution of these enzymatic activities.
We identified several genomic regions which control the emission of the terpenes DMNT, TMTT, and nerolidol, and encode potential regulatory elements like transcription factors. Transcriptome analysis identified several transcription factors that display herbivore-controlled expression patterns. Functional characterization of one of these transcription factors with maize transposon insertion mutants indicated that it is specifically involved in the regulation of DMNT and TMTT production after herbivory.

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T20

Mycorrhizal phosphate uptake affects maize root-associated microbiota

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The crop model maize (Zea mays L.) thrives by interacting with an astounding number of microorganisms, i.e. the so-called microbiota, which comprises mainly bacteria, but also fungi, protozoa and viruses. In most plants arbuscular mycorrhizal fungi (AMF) are an important functional group of terrestrial microbiota; they are beneficial for maize growth and health on agricultural soils with low phosphate (Pi) availability. Little is known about the drivers of microbial community assembly in the mycorrhizal root.
Maize plants colonized by AMF Rhizophagus irregularis exhibited systemic alterations in their leaves, including anthocyanin and lipid metabolism, changes in metabolic fluxes, but also induction of defense gene expression and accumulation of secondary metabolites suggesting priming of mycorrhizal maize leaves (Gerlach et al., 2015). We thus hypothesized that alterations at the root level as a consequence of AM colonization and symbiotic functions impact the structure of the root-associated microflora.
A transposon insertional maize mutant defective in Pi transporter Pht1;6 was impaired in mycorrhizal Pi uptake and exhibited strongly reduced colonization by AMF when grown in isolation, and a strong reduction of shoot growth and cob development. This highlighted the importance of mycorrhizal Pi uptake for maize performance. Moreover transcriptomic analysis of mycorrhizal wild type relative to pht1;6 via RNAseq revealed differential regulation of genes involved in signalling, hormone metabolism, and cell wall biosynthesis. In addition, elemental fingerprinting revealed an altered elemental composition in pht1;6 shoots. When cultivated in community with wild type plants colonization by AMF was restored in pht1;6 (Willmann et al., 2013). Such trans-complementation assays highlighted the impact of neighboring mycorrhizal plants on the root-associated microbiota of pht1;6. Using methodology to sequence and explore soil- and root-inhabiting microbial communities by 16S rRNA and ITS surveys, we now show that Pht1;6 activity is a driver of the taxonomic structure of fungal and bacterial assemblages.

Gene / Gene Models described: *ZEAma;PHT1;6*; GRMZM5G881088_T01
Funding acknowledgement: German Ministry of Education and Research, International Max Planck Research School Cologne

T21

Discovery of dolabralexins, previously unrecognized terpenoid defense compounds in maize (*Zea mays*).

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Specialized terpenoids are major components of complex maize (*Zea mays*) chemical defenses that mediate responses to herbivores, pathogens and other environmental challenges. Here we describe the discovery, biosynthesis and elicited production of a new class of maize diterpenoids, named dolabralexins. Metabolite profiling of common maize cultivars under field conditions supports the widespread biosynthesis of dolabralexins as predominant metabolites in roots. Oxidative stress and elicitation with fungal Fusarium pathogens elicit the accumulation of dolabralexins and the transcript expression of corresponding biosynthetic genes. Consistent with fungal-elicited defenses, select pathway intermediates significantly inhibit fungal growth in vitro. Furthermore, dolabralexin and previously described kauralexin diterpenoids are critical for determining the rhizosphere microbiome composition. Together, these findings support defense-related roles for dolabralexins in maize stress interactions and expand the known chemical space of diterpenoid defenses as genetic targets for understanding and ultimately improving maize resilience.

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T22

Predicting Across the Central Dogma of Molecular Biology: DNA to mRNA Abundance

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The central dogma of molecular biology is a framework for understanding how genetic information results in organismal level phenotypes. Being able to predict one step along the central dogma from its previous step would enable greater understanding of basic biology and advances in crop improvement. To do this, we created a number of machine learning models that take as input genomic regions upstream of coding sequences. Initially, we used modified natural language processing algorithms (bag-of-words, and word2vec) to model chromatin domains and transcription factor binding sites in the genome with aROC values greater than 0.97. Building on those models, we next developed convolutional neural network (CNN) architectures for the simple problem of predicting if a gene is a pseudo-gene. These models perform with an aROC above 0.87. We further designed CNN models which predict absolute gene expression values with an R2 of 0.49. Examination of these models show that they place considerable weight on both the 5' and 3' UTR regions. Some drawbacks of this model however are the potential for gene family-derived contamination in training and testing sets, and dataset imbalance. Additionally, for applied breeding one actually wants to know relative expression among alleles in a population, not necessarily their absolute expression values. To overcome these challenges we designed a predictive framework in which maize genes are compared with their syntenic orthologs in Sorghum and other close relatives. The CNN model then predicts which ortholog from the group is more strongly expressed. This model performs with aROC value above 0.90 and represent a first step in promoter strength prediction of maize alleles. These models have clear applications to cis-regulation, promoter function, gene annotation, and enhancement of genomic prediction.

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

T23

Mapping the archesporial cell to meiocyte progression using single-cell RNA-Seq

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Meiosis is one of the most dramatic changes in an organism's life cycle. In the maize anther, the first cell type committed to a meiotic lineage forms nearly a week before the onset of meiosis. These meiotic precursor cells undergo substantial changes during this time: increasing in volume 30 fold, developing a characteristic morphology with prominent nucleoli, and installing a subset of meiotic proteins onto the chromosomes. To gain a high resolution view of the developmental program leading up to meiosis, we used single-cell RNA-sequencing to follow changes in gene expression between the specification of the germinal lineage through the first few stages of meiotic prophase I. We identified three major phases of gene expression during this interval, with a particularly rapid rearrangement of the transcriptome shortly before the onset of meiotic chromosome pairing. Our data suggests substantial coordination between cytoplasmic remodeling and chromosomal reorganization. We are currently investigating the functional importance of rapid new transcription during the beginning of meiosis.

Funding acknowledgement: National Science Foundation (NSF)

T24

Single parent expression is a general mechanism driving extensive complementation of non-syntenic genes in maize hybrids

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Distantly related maize (*Zea mays* L.) inbred lines exhibit an exceptional degree of structural genomic diversity, which is probably unique among plants. We surveyed how the structural genomic diversity of a maize inbred line panel (B73, Mo17, A554, H84, H99, Oh43 and W64A) affects the transcriptomic plasticity of their F_1-hybrids during three stages of early primary root development. A RNA-seq experiment was designed to maximize the number of direct comparisons among the parent-hybrid pairs and to simultaneously ensure a high degree of precision for indirect comparisons. Genes active in one but inactive in the second parental inbred line represent an extreme instance of allelic diversity, which was denoted as single parent expression (SPE). We demonstrated that extreme gene expression complementation in F_1-hybrids is a general mechanism extensively implemented by genes active in only one parent. In all genotype-by-stage combinations ~1,000 genes show SPE patterns even in B73-independent hybrid crosses of the distantly related inbred lines Oh43 and W64A. Along primary root development, a substantial number of genes displaying SPE patterns were conserved, while only a small proportion were conserved between the different genotypes. Consequently, the number of expressed genes in all hybrids at all developmental stages exceeded their parental inbred lines by several hundred. Gene expression complementation is mainly driven by evolutionary younger non-syntenic genes, which emerged after the separation of the maize and sorghum lineages. Among those, the highly diversified families of bZIP and bHLH transcription factors were specifically overrepresented. Based on their attributed functions, these genes individually provide only minor advantages, but might collectively contribute to the superior plasticity of hybrids.

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

T25

The regulatory landscape of developing maize inflorescences: linking phenotypic variation to the functional non-coding genome

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Gene regulation has a profound effect on developmental plasticity contributing to plant adaptation and yield potential. Although many key protein coding genes (PCGs) have been identified that control intricate aspects of maize development, little is known about how the non-coding space, including long non-coding RNAs (lncRNAs), *cis*-regulatory elements and transposable elements, contributes to fine tuning of developmental processes. To define the functional landscape of early maize inflorescence development, we generated genome-wide chromatin accessibility maps using micrococcal nuclease sensitivity coupled with high-throughput sequencing (MNase-seq) in young tassel and ear primordia. We annotated potentially active regulatory regions in these tissues by applying a segmentation algorithm called ISeg, and by integrating publicly available datasets.
Depending on the peak-calling stringencies used, our analyses showed that 1.6-4.8% of the maize genome was accessible during early inflorescence development and approximately 80% of hypersensitive regions were shared between tassel and ear. Hypersensitive regions were found in various genomic contexts; enriched within proximal promoters and in the 3' UTRs and non-coding space immediately downstream of PCGs. To better understand the role of non-coding functional elements, we annotated 2,760 high-confidence lncRNAs using developmentally staged ear and tassel RNA-seq data. These lncRNAs were largely within 10 kb of PCGs, overlapped accessible chromatin regions, and were highly expressed in inflorescence primordia. Approximately 5% of these high-confidence lncRNAs were conserved between maize and sorghum. In addition, we integrated GWAS analyses for inflorescence architecture traits and showed enrichment of trait-associated SNPs in accessible regions, which were used to further prioritize functional elements that contribute to regulation of inflorescence development and variation in phenotype. Together these data provide genome-scale insight into the coordinated regulation of gene expression during inflorescence development, and an untapped source of allelic variation that can be leveraged for future breeding programs.

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T26

Circular RNAs mediated by transposons are associated with transcriptomic and phenotypic variation in maize

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Circular RNAs (circRNAs) are covalently closed RNA molecules. Recent studies showed that circRNAs can arise from the transcripts of transposons. Given the prevalence of transposons in the maize genome and dramatic genomic variation driven by transposons, we hypothesize that transposons in maize may be involved in the formation of circRNAs and further modulate phenotypic variation. We performed circRNA-Seq on B73 seedling leaves and uncovered 2,804 high-confidence maize circRNAs, which show distinct genomic features. Comprehensive analyses demonstrated that sequences related to LINE1-like elements (LLE) and their Reverse Complementary Pairs (LLERCPs) are significantly enriched in the flanking regions of circRNAs. Interestingly, as the number of LLERCPs increase, the accumulation of circRNAs varies, while that of linear transcripts decreases. Furthermore, genes with LLERCP-mediated circRNAs are enriched among loci that are associated with phenotypic variation. These results suggest that circRNAs are likely to be involved in the modulation of phenotypic variation by LLERCPs. Further, we showed that the presence/absence variation of LLERCPs was associated with expression variation of cicrcRNA-circ1690 and was related to plant ear height potentially through the interplay between circRNAs and functional linear transcripts. Our first study of maize circRNAs uncovers a potential new way for transposons to modulate transcriptomic and phenotypic variations.

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T27

Dynamics of DNA methylation during maize reproductive development

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Maize is blessed with relatively large reproductive cells, and an exceptional cytology. It is thus a powerful experimental model to analyze chromatin dynamics during reproduction at high resolution, in a stage-specific manner, using a combination of methylome analysis, and hyper-resolution microscopy. We have developed efficient protocols to study DNA methylation using bisulfite sequencing in isolated reproductive cells, in both wild type plants and mutants affected in DNA methyl-transferase activity. We have generated a temporal series of methylomes covering individual stages of male meiosis, including prophase I (leptotene, pachytene, dyakinesis), metaphase I, dyades, prophase II, tetrads and young spores. The data shows that DNA methylation during meiosis is dynamic, and significantly different from the patterns observed in somatic cells. We further looked at the dynamic of methylation in developing embryos. We uncovered a rapid process of hyper-methylation specifically in the CHH context, which is strictly dependent on RNA-directed DNA methylation. DNA methyl-tranferase mutants in maize display a number of developmental effects, including strict embryo lethality for CG methyltransferases, but also distinctive effects on meiosis, gametogenesis and embryogenesis for mutants affecting CHG and CHH methylation. We are currently analyzing the functional bases of these phenotypes using both classical cytology, bisulfite sequencing and hyper-resolution microscopy. The data shows that Zmet5, a maize homologue of Arabidopsis CMTs, is a key player of meiocyte methylation at non-CG sites. The mutant shows high degrees of sterility, linked to clear meiotic abnormalities. Altogether, the data indicates that maize represents a remarkable model to establish causal relationships between DNA methylation patterns and reproductive functions.

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T28

Transposable element contributions to the dynamic maize genome and transcriptome

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Transposable elements (TEs) comprise the majority of the maize genome and have the potential to contribute substantially to structural and expression variation among genotypes. Our understanding of the consequences of variable TE insertions has been limited by challenges associated with assembling and analyzing repetitive sequences genome-wide. The recent availability of high-quality de novo genome assemblies for W22 and PH207, along with a structural annotation of TEs, provides the opportunity for genome-wide analysis of the contribution of TEs to genome structural variation in maize. For over 150,000 TEs present in the B73 genome, we used flanking sequences to classify elements as conserved or polymorphic in PH207 and W22. Over 700 Mb of the B73 genome assembly consists of TE insertions that are absent from the W22 assembly. We also find differences in DNA methylation among lines at polymorphic sites, demonstrating a genetic contribution to epigenetic variation. In addition to creating structural variation, TEs also affect the transcriptome through their own expression or through effects on nearby genes. Using a novel approach to monitor the expression of TE families using unique and multiply-mapping RNA seq reads, we show that ~5% of the transcriptome is derived from TEs in most tissues. A targeted analysis of RNA seq data from maize seedlings subjected to abiotic stresses such as heat, cold, salinity or drought revealed hundreds of TE families and thousands of genes that were up- or down-regulated. Many genes with altered expression are located near up-regulated TEs suggesting a possible regulatory influence of TEs on nearby genes, a model that we are testing in genotypes with variable TE insertions. The analysis of shared and polymorphic TE insertions in multiple maize genomes will be critical for defining the role of TEs in creating genetic, epigenetic and gene expression variation in maize.

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

T29

The evolutionary dynamics of maize small RNAs reveals novel regulatory features in noncoding DNA.

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The majority of the maize genome is composed of transposon-derived repeats. The contribution of these noncoding sequences to regulatory variation and phenotypic traits is difficult to assess because they are not effectively captured by SNPs and are subject to epigenetic control. Small RNAs (sRNAs) modulate chromatin and transposon silencing via both RNA-directed DNA methylation (RdDM) and RNA interference. To gain insights into the activities of noncoding DNA, we sequenced sRNAs from diverse populations of maize inbreds, their corresponding hybrids, and pedigrees derived from crosses of divergent inbred parents. Multiple modes of sRNA variation were observed that exhibit distinct population structures compared to SNPs, and suggest genome-scale regulatory function. Short (21-22nt) sRNAs active in RNA-interference of transcribed transposons show rapid and dynamic evolution compared to long (24nt) sRNAs associated with heterochromatic silencing. We find wide variation for the abundance of 24nt sRNAs clustered within 200-bp of the boundaries of some maize genes, which coincide with islands of CHH-methylation, open chromatin, and stronger mRNA expression. We discovered these "gene boundary sRNAs" are always highly abundant among inbreds from the Stiff Stalk heterotic group, but are variable and less abundant in Non-Stiff Stalk inbreds. Integration of sRNA variation with RNASeq expression profiles and phenotypic data using WGCNA regulatory network approaches identifies functional modules associated with grain yield. One such module contains the RLG00010 transposon recently discovered to be enriched in transcriptional enhancers. Another reveals that phasiRNAs, which are required for male fertility in cereals, are also present in the developing ear and are negatively correlated with grain yield. We conclude that sequencing sRNAs reveals novel genome-scale regulatory properties that are not evident from DNA-trait associations, yet can be associated with important productivity traits.

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T30

Chromatin as a major determinant in the fate of meiotic double-strand breaks and positioning of COs along maize chromosomes

(submitted by Adele Zhou <az266@cornell.edu>)

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Most crossing-overs (COs) in maize, and other large-genome plants, are located near chromosome ends, while few are in pericentromeric or centromeric chromosome regions. As ~20% of maize genes are in the recombination-suppressed regions, this pattern presents a serious challenge to plant breeding. To solve this problem, we study recombination pathway dynamics. COs are products of the meiotic recombination pathway that is initiated by programed double-strand breaks (DSBs) in chromosomal DNA. Although the CO distribution is biased, DSBs, which are much more numerous than COs, are present in maize in all chromosome locations, including pericentromeric and centromeric regions. Previous studies have linked meiotic recombination landscape to chromatin features, but it is not well understood how this interaction is regulated and how it affects which of the many DSBs become COs. To address this question, we elucidated the genome-wide relationship between DSBs, COs, chromatin marks H3K4me3, H3K9me2, and DNA methylation. We found that DNA methylation is a major contributor to the decision of which DSBs become COs and this decision is progressively enforced when meiotic DSBs are repaired. Interestingly, this process proceeds differently in B73 and CML228, two maize inbreds with dramatically differing numbers of recombination events. The relationship between DSBs, COs, and chromatin state is altered when DNA methylation levels are reduced in the *zmet2* mutant. In *zmet2*, CO numbers are increased overall and CO distribution is dramatically shifted, with the CO number increasing in pericentromeric regions, at the expense of chromosome ends. Thus, the *zmet2* mutant can be used to facilitate recombination in pericentromeric regions of maize chromosomes. We propose that a reduction in DNA methylation opens up CO formation to regions of the genome that were previous blocked by DNA methylation.

Funding acknowledgement: National Science Foundation (NSF)

T31

Dissecting a new connection between cytokinin and jasmonic acid in control of leaf growth

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Growth is critical for multicellular plant development and underlies many important agronomic traits. A comprehensive understanding of the interacting signals that fine-tune plant growth, such that it is balanced with other physiological responses, has yet to be fully realized. We are using the maize leaf as a model to better understand the signals regulating plant growth, since the two cellular processes driving growth – division and elongation – are spatially separated into distinct zones at the leaf base. Our analysis of the semi-dominant *Hairy Sheath Frayed1* (*Hsf1*) mutant indicated it had a smaller cell division zone and reduced leaf growth caused by hypersignaling of the phytohormone cytokinin. Cytokinin (CK) typically functions to promote cell proliferation but can also repress growth in certain contexts. How CK mediates repression is not well-defined. Our analysis of *Hsf1* revealed that the mutant over accumulates jasmonic acid (JA) in growing leaves, a hormone previously shown to both repress cell division and activate defense pathways. This result suggested CK may crosstalk with JA in the control of leaf growth. Such a connection was previously unrecognized and may explain one route by which CK can repress growth. To investigate this connection, we determined that exogenous JA application repressed leaf growth while JA-deficiency increased leaf growth in maize. We also assessed JA pathway gene expression levels in the division and elongation zones of emerging leaves of *Hsf1*/+ and wild type (WT) seedlings. Several JA biosynthesis and responsive genes were significantly upregulated in the growth zone of *Hsf1* mutants compared to WT sibs. JA-pathway gene expression was also induced in B73 inbred seedlings transiently treated with CK. Overall, our results suggest CK signaling promotes JA accumulation through up-regulation of JA biosynthesis. Further analysis of this new connection may provide insights into the mechanisms by which plants balance growth with other processes, such as defense response.

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T32

How grass keeps growing: a predictive model of leaf growth regulation based on studies in Zea mays

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Due to its size maize is an excellent model system to investigate the regulation of leaf growth in grass species. Here we propose a mathematical model based on hormone cross-talk with auxin and cytokinin as the primary signals determining basal rate and duration of growth. On top of that platform active gibberellins provide an additional layer largely controlling growth rate, in a DELLA dependent way, which may be vital for developmental plasticity and stress response. The model implemented with the modelling software VPTissue can reproduce steady maize leaf growth characteristics as well as spatial patterns of hormones and pathway enzymes in wild type, overexpression and dwarf lines. Importantly, the model predicts temporal changes in hormone regulation such as a standing and then retreating GA 1/8 wave and how this affects leaf growth. Deviation between model and experimental data suggest the existence of a a growth-driven negative feedback on growth termination, which could be experimentally validated by excision and shading of the emerged part of the growing leaf.

Funding acknowledgement: Flanders Science Fund (FWO), Belgian Science Policy Office (BELSPO)

T33

TREHALOSE-6-PHOSPHATE PHOSPHATASE4, a paralog of RAMOSA3, controls inflorescence architecture and shoot apical meristem activity in maize

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In recent years, the disaccharide trehalose-6-phosphate (T6P) has emerged as an important regulator of meristem function. A prime example of this is the classical maize mutant *ramosa3* (*ra3*), which has branched ears and more highly branched tassels due to a reduction in meristem determinacy. The causal gene encodes a trehalose-6-phosphate phosphatase (TPP) that degrades T6P by converting it to trehalose. However, how RA3 regulates meristem function remains enigmatic.

In order to find additional factors involved in RA3 function, *ra3* was mutagenized to identify additional mutations that enhance its ear branching phenotype. This screen revealed four alleles of *tpp4*, mutated in a paralog of *RA3*. *TPP4* is expressed in the same domain as *RA3*, and its expression is upregulated in *ra3* mutants, indicating functional redundancy where *TPP4* partially compensates for loss of *RA3*. All mutant alleles have amino acid substitutions in conserved residues, resulting in a range of residual enzymatic activities. Nonetheless, all alleles have similar phenotypic strength and behave semidominantly, suggesting that there is no straightforward relation between TPP activity and phenotype. In agreement with this, no change in T6P levels could be detected in these mutants, adding to a growing body of evidence for regulatory functions for TPPs.

In the presence of functional *RA3*, *tpp4* mutants have no inflorescence architecture phenotypes, but show enlarged shoot apical meristems (SAMs). While the SAM is wider throughout development in *tpp4* mutants, SAM height is only significantly increased around the floral transition, suggestive of earlier flowering. Accordingly, floral transition markers are expressed earlier in *tpp4* SAMs, and there is a tendency towards earlier anthesis and silking in the field. These results suggest that the function of T6P in flowering time regulation, first reported in Arabidopsis, is conserved in maize, and further establish the importance of TPPs in the regulation of meristem activity.

Gene / Gene Models described: *TPP4*; Zm00001d052227
Funding acknowledgement: National Science Foundation (NSF), EMBO, DuPont-Pioneer

Poster Abstracts

P1

An expanded diversity panel reveals the magnitude of the maize pan-genome and limitations of a single-reference genome sequence for GWAS

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Recent work in plants, including maize, has illustrated the relative prevalence of core and dispensable genes within a given species. The goal of this work is to assess the impact of utilizing different maize genome references to identify sequence variation and the relative contribution of different components of the pan-genome to phenotypic expression. Previous work with a 503-member North American diversity panel of maize identified nearly 9,000 novel transcripts not present in the reference B73 RefGen_v2. To better capture the diversity available within temperate North American maize germplasm, we enlarged that diversity panel to include whole seedling RNA-seq from a total of 942 inbreds and generated pan-genomes in parallel using both the Zm-B73-REFERENCE-GRAMENE-4.0 and the Zm-PH207-REFERENCE_NS-UIUC_UMN-1.0 genomes as references for read alignment and subsequent SNP calling and expression profiling. A total of 34,447 and 39,672 novel transcripts were identified using the B73 and the PH207 reference genomes, respectively, comprising a fourfold increase in the number of novel transcripts compared to previous estimates. This suggests that the number of dispensable genes and the overall size of the maize pan-genome are much larger than previously thought. Genome-wide association studies of reproductive and disease resistance traits reveal associations between phenotypes and both core and dispensable genes. This finding highlights the importance of considering novel transcripts and multiple references for the detection of phenotype-associated variants that would otherwise be difficult or impossible to identify due to presence/absence variation in a traditional single-reference framework.

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P2

Both hard and soft sweeps contribute to local adaptation of natural populations of teosintes

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Maize was domesticated from the wild grass teosinte, *Zea mays* ssp. *parviglumis*. While much is known about the genetics of maize domestication and differences between maize and teosinte, we know relatively little about the evolution of teosinte in natural populations. To begin to understand the evolutionary forces shaping genetic diversity in teosinte, we sequenced the genomes of multiple wild-collected individuals from each of six natural populations spanning much of the geographic range of teosinte in southern Mexico. Here we present preliminary population genetic analyses of these samples. We find evidence of hard and soft selective sweeps mostly private to individual populations, suggesting considerable local adaptation. We also show variable differentiation and inbreeding among populations, consistent with hierarchical population structure and metapopulation dynamics. Overall, our results on the evolution of teosinte in its natural habitats will likely provide insight into the genome-wide patterns of diversity in locally adapting plants as well identify loci relevant for adapting maize to new and changing environments.

Funding acknowledgement: National Science Foundation (NSF)

P3

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

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

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

P4

Coming soon: Whole genome assembly of the maize NAM founders

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Maize is an important crop and model organism for plant genetics. However, currently, nearly all forms of sequence analysis are referenced to the single B73 inbred. Beyond B73, the most extensively researched maize lines are the core set of 25 inbreds known as the NAM founder lines, which represent a broad cross section of modern maize diversity. Prior data show that gene content can differ by more than 5% across lines and that as much as half of the functional genetic information lies outside of genes in highly variable intergenic spaces. To capture and utilize this variation, the NAM founder inbreds and a twenty-sixth line containing abnormal chromosome 10 will be sequenced and assembled using a high-throughput strategy. Scaffolds will be validated by BioNano optical mapping, and ordered and oriented using linkage data. RNA-seq data from multiple tissues will be used to annotate each genome, and assemblies and annotations will be released with genome browser support through MaizeGDB, NCBI, and Cyverse. Comparative genomic tools will be used to identify and catalog the maize pangenome, and assess the role of structural variation such as presence-absence variation and copy number variation in the determination of agronomic traits. Timeline and results will be disseminated through a project web site. This work will be supported by NSF award #1744001.

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P5

Comparing alternative splicing in maize using 4 different reference genomes

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Maize is a highly polymorphic species with extensive genetic diversity, gene copy number variation, and gene presence-absence variation between genotypes of inbred lines. The current reference genome used for maize bioinformatics studies is the genome of the B73 inbred line. Previous work has been conducted investigating alternative splicing (AS) dynamics in maize between tissues, as a result of stress responses, and between genotypes.
AS is a process enabling multiple transcript isoforms to be coded from a single gene thereby expanding an organism's proteome without increasing the size of the genome. Given the genetic diversity of maize, this study profiles the diversity and conservation of AS between 27 maize genotypes. However, instead of comparing these AS events between genotypes using only the B73 v4 reference genome, this study integrates the AS events discovered in these genotypes relative to the W22, PH207, and CML247 reference genomes. Currently all AS events occurring in these genotypes relative to these reference genomes have been discovered and validated. Preliminary results generated by comparing the AS events between genotypes relative to each reference genome and by comparing the AS events discovered within genotypes using different reference genomes.

Funding acknowledgement: National Science Foundation (NSF)

P6

Computational classification of phenologs across biological diversity

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Phenotypic diversity analyses are the basis for research discoveries that span the spectrum from basic biology (e.g., gene function and pathway membership) to applied research (e.g., plant breeding). Phenotypic analyses often benefit from the availability of large quantities of high-quality data in a standardized format. Image and spectral analyses have been shown to enable high-throughput, computational classification of a variety of traits across a wide range of phenotypes. However, equivalent phenotypes expressed across individuals or groups that are not anatomically similar can pose a problem for such classification methods. In these cases, high-throughput, computational classification is still possible if the traits and phenotypes are documented using standardized, language-based descriptions. In the case of text phenotype data, conversion to computer-readable "EQ" statements enables such large-scale analyses. EQ statements are composed of entities (e.g., leaf) and qualities (e.g., length) drawn from terms in ontologies. In this work, we present a method for automatically converting free-text descriptions of plant phenotypes to EQ statements using a machine learning approach. A random forest classifier identifies potential matches between phenotype descriptions and terms from a set of ontologies including GO (gene ontology), PO (plant ontology), and PATO (phenotype and trait ontology), among others. The features used by this classifier include semantic, syntactic, and context similarity metrics between words and ontology terms. This classifier is trained and tested using a dataset of manually converted plant descriptions and EQ statements from the Plant PhenomeNET project (Oellrich, Walls et al., 2015). The most likely matching terms identified by the classifier are used to compose final EQ statements with confidence scores. Results of evaluating the accuracy of this approach are presented, and potential use across datasets to enable automated phenolog discovery are discussed.

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P7

Deciphering molecular origin and functional impact of structural variation in maize through genome sequences comparison and integrative analysis of genetic variation, transcriptome and phenotype data.

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Structural variation (SV) is a major driver of plant adaptation and genome evolution. It originates from transposable element insertion, as well as gene Copy Number (CNV) and Presence/Absence Variation (PAV). Maize is a crop species with a complex genome, and exhibits extensive SV among lines, as well as strong phenotypic differences. It is therefore a good model to explore the diverse molecular mechanisms leading to SV, and to investigate to what extent SV impacts phenotypic variation. Finally, the geographical origin of the different maize inbred lines is well described, allowing for linking SV to environmental adaptation.
Here, we present whole genome assemblies from seven European and American maize lines of various geographical origins and phenotypes, and with contrasted genome size. This dataset allows unprecedented genome-wide comparisons and characterization of maize SV with high sequence accuracy, thus offering the opportunity to evaluate the prevalence of the molecular mechanisms underlying these variations and to characterize the features responsible for genome size variation.
These seven maize lines together with B73 were cultivated under contrasted water conditions in the PHENOARCH phenotyping platform allowing precise characterization of growth and development together with precise measurements of environmental conditions. Thirteen different organs harvested at various developmental stages have been used for RNA-seq-based transcriptome analysis. This massive dataset will be used to evidence the possible role of SVs in quantitative responses to water deficit as well as the impact of SVs in gene regulation networks. Overall, this work will provide insights on the molecular origins and functional consequences of SV.

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P8

Four European Flint reference sequences complement the maize pan-genome

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Compared to US Dent material, the European Flint germplasm represents a distinct group which is not well represented by Dent genomic resources. To gain insight in structural and functional differences between Flint and Dent genomes, we generated four de novo Flint reference sequences. We sequenced the inbred lines EP1 originating from the Spanish landrace –'Lizargarate', F7 from the French landrace 'Lacaune', DK105 from the German landrace 'Gelber Badischer Landmais', and doubled-haploid line PE0075 from the German landrace 'Petkuser Ferdinand Rot' at 214x to 320x coverage. Pseudochromosomes were assembled with NRGene's DeNovoMAGIC 2.0 or 3.0 technology, respectively, and total assembly sizes ranged from 2.2 to 2.5 Gb. For EP1 and F7 comprehensive RNAseq data are available to assist gene annotation. We made use of the PGSB maize repeat database (Spannagl et al. 2016, Nucl Acid Res) for repeat annotation). To investigate structural genomic variation, we performed pairwise whole genome alignments (WGAs) using the MUMmer software (Kurtz et al. 2004, Genome Biol) between EP1, F7, B73_v4 (Zm00001d) and PH207 (Zm00008a). Numerous insertions/deletions, inversions, and transclocations, were detected, ranging from several kb to Mb. Results of the WGAs and comparison with high-density genetic mapping data will be presented.

Beta-versions of the de novo assembled genomes, Zm-EP1-REFERENCE-TUM-1.0 (Zm00010a) and Zm-F7-REFERENCE-TUM-1.0 (Zm00011a), have been released in collaboration with NCBI and MaizeGDB under the Toronto Agreement for prepublication data sharing. The assemblies of DK105 and PE0075 will be released when annotation has been completed.

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P9

Functional analysis of maize kernel development

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High-throughput, long-read sequencing methods provide whole genome assemblies for molecular genetic analysis, including the analysis of EMS mutants by bulked segregants analysis (BSA). Although BSA is widely used to identify SNPs that are linked to phenotypes, it is more challenging for maize because of the proportion of transposable elements in its genome. Still, key mechanisms of maize kernel development could be derived from a classical *defective kernel* (*dek*) mutant collection, generated by Neuffer and Sheridan in the 1980s, if the sequences of these EMS mutations could be identified. However, these *dek* mutations were generated in genotypes, whose genome had not been sequenced. To locate these mutations in the genome, we introgressed the *dek* phenotypes into two inbred lines, by backcrossing all 30 *dek* mutants available from the maize stock center to both B73 and W22 four times. These inbreds have been selected because their genomes have been sequenced by single molecule, long-read sequencing platforms, which provide a better resource for such an approach than highly fragmented short-read whole-genome assemblies. We have selfed and collected bulked samples for each introgression and can now identify the molecular nature of each *dek* phenotype by either exome or RNA sequencing because of the divergence of B73 and W22 genome.

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P10

Genomic prediction using TensorFlow

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Genomic prediction has proven to be a powerful tool to increase the gain of selection in plant and animal breeding programs. A variety of statistical procedures are commonly applied to predict performance of untested genotypes, including GBLUP and a set of related algorithms known as the bayesian alphabet. In recent years machine learning algorithms were used interdisciplinary for prediction and classification. This was accelerated after Google published their own machine learning library Tensor Flow.
We used Tensor Flow, implemented in Python 3, to use probabilistic neural networks to predict performance of simulated data, maize landraces and *A. thaliana* phenotypes and compared the results to the performance of GBLUP and were able to show that our machine learning architecture was capable to outperform GBLUP in some cases. Furthermore we assessed the capability to predict within and across two landrace doubled-haploid populations derived from Petkuser and Kemater maize landraces. In the future it is planned to include environmental "markers" in those models to predict traits of interest of unknown genotypes in untested locations and to account for GxE

P11

Gramene maize pan-genome browser

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Maize is the most genetically diverse crop in the world, with differences in gene content estimated between 5-20% among lines. Capturing the pan-Zea gene space and structural variation requires additional reference genomes, and the infrastructure to store, analyze and make accessible. To support this effort, Gramene has developed a dedicated genus-level browser resource: **maize-pangenome.gramene.org**, built upon the Ensembl infrastructure and guided by FAIR practices. Our first pass of this resource includes B73, W22, and PH207 complete reference genomes, along with 7 monocot and dicot outgroup species. These served as input to generate phylogenetic resources based on protein and whole-genome DNA alignments. Insights into ancestrally conserved regions and structural rearrangements are defined by pairwise whole-genome alignments and displayed in a number of informative ways, including a multi-species view that allows graphical stacking of browsers and interspecies navigation. The gene trees can be used to programmatically identify gene expansions and losses between different maize accessions, which may explain evolutionary adaptations, inaccuracies in the gene models, or errors in the underlying reference genome assemblies. We anticipate maize accessions like the NAM populations being added to this resource. To test the utility of these resource and to assess quality of the gene structure predictions, Gramene outreach efforts include the first maize annotation jamboree co-organized with the MaizeCODE project. This work constitutes an initial prototype to support the infrastructure to identify misannotated gene structures and a process to correct these guided by the gene trees. In addition to providing resources to support quality assessment, as well as insights into many outstanding questions in the evolutionary history of the *Zea* genus, this resource will provide a basis for functional characterization of genes and the identification of targets for agronomic improvement of maize. This project is funded by NSF (IOS-1127112) and partially from USDA-ARS (1907-21000-030-00D).

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P12

Growth and metabolic responses of NAM parent lines to a brief exposure to cold during early seedling development

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Early planting can help prolong the growing season, thereby increasing yields, but this practice also makes it more likely that seedlings will be exposed to cold stress. Depending on the underlying genetics, early cold stress, even in short bouts, can impact adult phenotypes. Therefore, the processes underlying cold tolerance in early development are useful targets for crop improvement. Identification of seedling biomarkers associated with the effects of early cold stress on adult growth would make it possible to more efficiently identify genetic factors that contribute to cold resistance. Beneficial microbes aid in protecting plants against abiotic stresses including temperature stress and chemical exudates are key signals facilitating this relationship. An objective of this study was to explore how root exudate composition varies by genotype before and after a short bout of cold stress delivered at the seedling stage in twelve NAM parent lines. Seedlings were germinated on paper and grown in agar tubes. After three days, a 24 hour cold stress treatment at 10°C was given to half of the seedlings. After two additional days of incubation at the control temperature, seedlings were removed from the agar tubes and were transferred to peat pots where they hardened for a week before transplanting to the field. Water soluble compounds were extracted from the agar remaining in each tube and metabolic fingerprints have begun to be collected from each sample using NMR spectrometry. Shoot growth, plant biomass and root morphology measurements were collected from each individual. Genotypes were found to vary in their adult growth in response to the seedling cold stress treatment. Seedling root digital biomass and growth rate are being used to normalize NMR spectra. Spectral regions differing by genotype and cold treatment are under investigation.

Funding acknowledgement: National Science Foundation (NSF)

P13

High level analysis of W23 and A619 genomes (sequenced by Novogene) compared to B73 reference

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Novogene generated paired-end 150-nt reads for W23 and A619 inbred lines on an Illumina HiSeq machine. Sequences were evaluated for quality, deduped, aligned to B73 using BWA mem (Li et al; v0.7.8), and processed for SNPs using Samtools (v 0.1.19) mdup. Based on the resequencing data variant calls, pseudo-reference genomes were generated using GATK (v3.6) FastaAlternateReferenceMaker. We compared the genomes and provide a high level analysis of the results. We show an overview of the process and are working with Novogene to streamline and improve a few of the data analysis and presentation steps. Variant Call Format (VCF) files for each inbred will be made available on Gramene's genome browser.

Funding acknowledgement: National Science Foundation (NSF)

P14

High resolution temporal and spatial transcription atlas of maize.

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Maize is an important model species and a major constituent of human and animal diets. To understand how the underlying genome sequence results in specific plant phenotypes, information on the temporal and spatial transcription patterns of genes is crucial. Here we present a comprehensive atlas of global transcription profiles across developmental stages and plant organs. We profiled transcript levels using RNA samples from 950 diverse tissues representing 5 major organ systems and varying developmental stages of the maize plant. The organ systems included root, stem, leaves, tassel, ear and seed. Each tissue was represented by three biological replicates. The developmental stages included day after sowing1, 2, 3, 4, 5, 6, seedling, trefoil stage, four leaf stage, elongation stage, panicle differentiation stage, flare opening, tasseling stage, days to silking, grain filling, milk repeness stage and dough stage.

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P15

Maize *Ufo1* mutant plays a role in epigenetic regulation and alternative splicing

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The maize *Unstable for orange1* (*Ufo1*) is a dominant mutation that gives rise to ectopic phlobaphenes accumulation in various tissues and shows pleiotropic growth defects. The *Ufo1* presence is accompanied by overexpression and hypomethylation of the reporter gene *pericarp color1* (*p1*), an R2R3 Myb transcription factor involved in the regulation of phlobaphenes biosynthesis. The *Ufo1* mutant also exhibits poor penetrance and low expressivity, and in order to better understand *Ufo1*'s role in epigenetic gene silencing, growth development and various stress response, we have performed RNA sequencing, small RNA sequencing and whole genome bisulfite sequencing from pericarp tissue.. Around 3000 (14%) genes were found to be differentially expressed (DE) (FDR < 0.05) in the mutant whereas around 800 genes were differentially methylated either in the promoter or gene body region and around 6% of the de novo identified small RNA loci were DE. A strong association was discovered between 24-nt small RNA expression and DNA methylation. Furthermore, we discovered that *Ufo1* mutation inhibits RNA splicing (intron retention in particular). Gene ontology analysis showed that the differentially spliced genes are enriched in GO terms for nucleotide binding/ATPase activity. Interestingly, we also found that a subset of alternative splicing events is affected by changes in DNA methylation at the splice sites. Overall, our analysis indicates that *Ufo1* mutation perturbs a subset of small RNAs and that DNA methylation is affected in gene bodies, which in turn may alter alternative splicing.

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P16

Functional divergence in maize subgenomes

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The maize organism experienced a whole genome duplication event approximately 5-12 million years ago. Since this event occurred after speciation from sorghum, the original genomes can be reconstructed by mapping syntenic regions to the sorghum chromosomes. Evolution in maize has been shown to result in uneven gene loss between each of the ancient genomes. This fractionation and divergence between these genomes continue today, influencing agronomic traits. Loss of one gene of a pair of homeologs can be explained as selective pressure favoring one copy of the gene and rendering the other unnecessary. Among those genes where both homeologs remain is the possibility for not only functional loss of one of the gene pairs, but generation of novel functions. Here we regenerate the subgenome reconstructions for the current B73 RefGen v4 genome assembly that were originally described for B73 RefGen v2. We find that the primary subgenome has a greater range of GO assignments, and that there is a relative lack of overlap between the subgenomes in terms of GO than would be suggested by expression and abundance data. By comparing both expression and abundance measures for these gene pairs across multiple tissues, we observe functional divergence of these homeologs. Although the primary maize subgenome is often expressing more highly than the secondary homeologs, we observed tissue-specific cases where the higher expressing homeolog belongs to the secondary subgenome.

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P17

MaizeGDB: stewardship for maize genome assemblies and annotation

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MaizeGDB is the genetics and genomics database for the model organism and agriculturally important crop Zea mays. One of the main priorities at MaizeGDB is to provide genome assembly and annotation stewardship for the maize research community. With falling sequencing costs and improved genome assembly methods, it has become feasible to generate dozens of reference-quality genome assemblies for maize accessions of importance to maize breeders and researchers. MaizeGDB currently hosts information for 10 high-quality genome assemblies (*Zea mays ssp. mexicana*, B104, B73, CML247, EP1, F7, Ki11, Mo17, NC350, PH207, W22, and others) and has integrated them with data held by MaizeGDB. This enables both exploring individual genomes, and comparing them in sets. In anticipation of more genomes expected in the near future, MaizeGDB developed a set of minimum standards for adopting a new genome assembly, designed templates for collecting essential metadata related to the genome and assembly, enforced naming conventions set out by the maize nomenclature committee, created documentation to help submit genome assemblies to GenBank, and developed a pipeline for loading new assemblies. All of this enables comparative analysis. In addition to bringing in new genome assemblies and providing the research community with means of improvement, MaizeGDB will continue stewardship of the B73 genome assembly and annotation, which is expected to remain the representative reference maize genome assembly for the foreseeable future. Multiple, high-quality genome assemblies and annotations integrated with trait, phenotype, and germplasm data, will improve researchers' ability to conduct trait and germplasm analyses and to choose appropriate germplasm for breeding programs.

Funding acknowledgement: United States Department of Agriculture (USDA)

P18

MaizeMine: a data mining warehouse for MaizeGDB

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MaizeMine (http://maizemine.maizegdb.org), the new data mining warehouse for MaizeGDB, accelerates genomic analysis by enabling researchers without scripting skills to create and export customized annotation datasets merged with their own research data for use in downstream analyses. MaizeMine uses the InterMine data warehousing system to integrate genomic sequences from the B73_RefGen_v3 and B73_RefGen_v4 genome assemblies, three sets of gene annotations (AGPv3, AGPv4, RefSeq), Gene Ontology (GO), protein annotations (UniProt), protein families and domains (InterPro), homologs (Ensembl Compara), pathways (CornCyc, KEGG, Plant Reactome), and single nucleotide variants (dbSNP). MaizeMine also provides pre-computed variant effects and expression levels based on RNA-seq data from the Zea mays Gene Expression Atlas (NCBI BioProject PRJNA171684). Database cross references facilitate easy gene identifier conversion between AGPv3, AGPv4 and RefSeq. MaizeMine provides simple and sophisticated search tools, including a keyword search, built-in template queries with intuitive search menus, and a QueryBuilder tool for creating custom queries. The Genomic Region search tool executes queries based on lists of genome coordinates, and supports both B73_RefGen_v3 and B73_RefGen_v4. The List tool allows users to upload identifiers to create custom lists, perform set operations such as unions and intersections, and execute template queries with lists. When used with gene identifiers, the List tool automatically provides gene set enrichment for GO and pathways, with a choice of statistical parameters and background gene sets. MaizeMine is particularly useful for tracking gene identifiers across gene sets to facilitate meta-analysis. Query results can be downloaded in several formats (tab delimited, GFF3, Fasta, BED, JSON, and XML).

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

P19

Making YOUR data and databases FAIR; Functional gene annotation and more!

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At MaizeGDB we value your input as we work towards making the best resource possible for you! Here we report new strategies for functional gene annotation through literature curation, suggestions for standard nomenclature, and new features on the gene model pages. We also have suggestions for you to make your data comply with the FAIR principles (https://www.force11.org/group/fairgroup/fairprinciples, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4792175/). We present the steps AgBioData member databases are now taking to make all public agriculturally related databases more interoperable and easy to navigate. At this poster, we are also soliciting your suggestion for improved content and organization. Please stop by!

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P20

MaizeGDB: new resources for maize researchers

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MaizeGDB, the USDA-ARS maize genetics and genomics database, is a highly curated, community-oriented informatics service to researchers focused on the crop plant and model organism Zea mays. MaizeGDB facilitates maize research by curating and maintaining a database that serves as the central repository for the maize community. With the availability of more reference quality genomes for maize, MaizeGDB has become more sequence-centric, while still maintaining traditional maize genetics datasets. The research focus of the maize community has continued to evolve, making it necessary to continually redefine data access and data analysis tools. In this poster we present an overview of new services and data types provided by MaizeGDB. New genome sequences are incorporated into MaizeGDB and made accessible through the annotation/assembly pages, BLAST databases, and genome browsers. Recently added genomes include B73v4, W22v2, Mo17, Mo17-Yan, PH207, CML247, B104, EP1, F7, Ki11, and NC350. MaizeGDB is responsible for stewardship of the maize representative genome assembly (B73), including the improvement of associations between the B73 gene models and gene models for all other assemblies. New resources include CornCyc 8.0, a tool allowing users to query metabolic pathways on the B73v4 assembly and SNPversity, a tool allowing users to compare SNPs across a diverse set of inbred lines. New tools under development include MaizeMine (an InterMine instance), MaizeDIG (a tool for tagging phenotypes in images and linking them to genes), and PedNet (a tool for visualizing pedigree networks).

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P21

Mining maize with Gramene

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Have you ever needed to know if the maize gene you work on has an ortholog in sorghum, or Arabidopsis? Has the gene family that you are working on expanded or contracted relative to other crop or model grass species? Is the biochemical pathway you work on conserved in sorghum and soybean? If so, you may want to explore these questions in the Gramene database. Gramene (**http://www.gramene.org**) is an integrated resource for comparative functional analysis in plants. Gramene provides researchers with access to 53 genomes, and pathways for 75 plants species, including *Zea mays* B73 (maize). Gramene builds upon Ensembl and Reactome software, and is committed to open accesses and reproducible science based on the FAIR principles, providing both human and machine access to the data. Gramene provides integrated search capabilities and interactive views to visualize gene features, gene neighborhoods, phylogenetic trees, gene expression profiles, pathways, and cross-references. Powerful phylogenetic approaches, including protein-based gene trees with stable IDs and whole-genome DNA alignments, enable traversing between maize and other plant species. Gramene hosts curated rice pathways, and uses these curated pathways to generate orthology-based projections for other species. Maize data includes the hosting of the maize RefGen_V4 assembly includes: i) functional descriptions for ~30K genes, ii) sub-genome designation and ohnologs, iii) transposable elements, and iv) V3-V4 gene ID conversion table and assembly converter to lift over genomic coordinates between V2, V3, and V4. Variation data includes the Panzea's 2.7 GBS (~720K SNPs in 16,718 lines) and maize HapMap2 (~55 million SNPs in 104 lines) data sets. Visualizations of EBI Expression Atlas data are integrated into the search results panel, and both genome and pathway browsers. Other annotation tracks include methylome signatures, genome-wide long non-coding RNAs, and nascent transcriptomes. Gramene is supported by an NSF grant IOS-1127112, and partially from USDA-ARS (1907-21000-030-00D).

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P22

MOA (MNase Open Access) mapping: A new and efficient method for genome-wide open chromatin profiling in maize, demonstrated with developing earshoots

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Chromatin structure is dynamic and intimately linked to gene regulation and other genetic functions. Our lab has developed a nuclease sensitivity profiling method (DNS-seq) that reveals sites of open chromatin linked to biological traits such as growth and yield (Rodgers-Melnick et al,. PNAS, 2016). Here we set out to develop a new MNAse-based open chromatin mapping technique (MOA) based on isolation and sequencing of small, subnucleosomal-sized fragments from light nuclease digests of chromatin from formaldehyde-fixed nuclei. Size selected libraries (~50-130 bp genomic fragments) sequenced at ~20 million reads per sample efficiently highlight open chromatin regions. We have found that these "small, light-digest" fragments produce many of the MNase-Sensitive Footprint signals from differential nuclease sensitivity (DNS) mapping (requiring over 300 million reads/replicated sample). We carried out MOA mapping on field-grown earshoots staged by size as 0-1 cm, 1-2 cm, 2-3 cm, 3-5 cm. One of these, the 1-2 cm earshoots (FES on GenoMaize browser), is one of the core reference tissues that has been fully profiled by DNS-seq (NSF PGRP project IOS 1444532). We will map the reads to the B73 genome and segment the coverage peaks using iSeg. Comparative analysis of DNS vs. MOA will improve our interpretation of each and allow us to test our prediction that MOA will uncover sub nucleosomal regions with developmentally variable occupancy. Experimental advantages of MOA-seq include the small amount of tissue required (< ~ 1g), the ability to use frozen tissues allowing for flexibility in harvest scheduling, and the relatively small number of sequence reads required. As a result, this innovation adds to the methods for identifying and monitoring activity of maize regulatory regions compared across multi-samples. Epigenomic profiling data will further improve our understanding of plant genome structure and function.

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P23

New software to identify and explore the orphan genes of maize

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The premise that new genes can arise from non-genic DNA sequences, borne out from massive sequencing data, sharply contrasts with the long- accepted view that novel gene functions primarily arise from a slow process of accumulated mutations and rearrangements of already-established genes. We hypothesize that a major role of orphan genes is to enable evolutionary adaptation to new environments. Because orphan genes have no homologs in other species, many current tools do not identify them efficiently. We describe new softwares designed to identify and delineate the genomic context of orphan genes, and to develop hypotheses as to the possible function of each gene. As maize genomes are sequenced, the orphan genes of these genomes can be categorized in the context of the adaptation and selection as the result of evolution and of human intervention for improved agronomic traits. We apply the tools to a systematic analysis of orphan genes across maize races and lines, and describe the insights and challenges from this comparative genomic analysis.

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P24

Organ-specific maize metabolic models from ensemble modelling

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Different plant organs show markedly different transcriptomics, proteomics, and metabolomics profiles largely due to differences in the underlying cellular networks. Here, we focus on reconstruction of organ-specific metabolic models for maize (i.e., root, shoot, and leaf) by integrating various omics-data with stoichiometric genome-scale metabolic models. Several computational strategies for extraction of tissue- and organ-specific models have already been proposed [1], and their performance in plants tested in our group for the case of A. thaliana [2]. Our main result consists of designing a combined modeling approach which uses the different computational methods for model extraction, aimed at leveraging the advantages of the existing solutions to generate an ensemble of consensus models. We validate the generated models against pathway-based evidence from various sources to select models with high predictive accuracy. We demonstrate that the ensemble modeling approach results in more specific and more predictive functional models than those obtained by the individual approaches. The models provide the prerequisite for combining the organ- and tissue-specific models into a functional multi-organ model.

[1] S. Robaina-Estévez, Z. Nikoloski (2017) On the effects of alternative optima in context-specific metabolic model predictions. PLoS Comput Biol 13(5): e1005568. doi:10.1371/journal.pcbi.1005568
[2] S. Robaina-Estévez, D. M. Daloso, Y. Zhang, A. R. Fernie, Z. Nikoloski (2017) Resolving the central metabolism of Arabidopsis guard cells. Scientific Reports 7 (8307) doi:10.1038/s41598-017-07132-9

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P25

Parallel transposase tagging (PTT-seq) technology is a cost-effective alternative for traditional Sanger sequencing

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DNA sequencing is fundamentally important for the genetics of all species. The traditional sequencing technology called Sanger sequencing was developed 40 years ago. Over the last decade, tremendous advances have been made by introducing the second or third generation sequencing technologies like the Illumina, Pacbio or Nanopore sequencing, with the cost of sequencing per base pair rapidly decreased. However, all the next generation sequencing technologies have a common feature -- sequencing millions of DNA fragments all together, and being not able to track the identity of individual fragment. Therefore, the traditional Sanger sequencing is still broadly used around the world by being able to obtain the sequencing information of any given individual fragments. Here we present our newly developed parallel transposase tagging (PTT-seq) technology. The PTT-seq is based on our novel strategies of barcoding the hyperactive Tn5 transposons so that the subclone libraries of each individual plasmids to be sequenced can be generated parallelly in high-throughput format. The subclone libraries of individual plasmids can be pooled together and then be sequenced using any of the next generation sequencing platform. Implementation of our PTT-seq technology can result in dramatic cost reduction as comparing to traditional Sanger sequencing. As an example, over 10,000 maize seed cDNA clones were sequenced rapidly with minimum cost while the identities of each individual clones correctly tracked, demonstrating that the PTT-seq can be a cost-effective alternative for traditional Sanger sequencing in many sequencing/genotyping applications.

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P26

Predicting crop leaf parameter from leaf reflectance spectra

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Harnessing natural variation in photosynthetic capacity is a promising route towards yield increases, but physiological phenotyping is still too laborious for large-scale genetic screens. Here, we evaluate the potential of leaf reflectance spectroscopy to predict parameters like specific leaf area, chlorophyll content, C/N ratio and photosynthetic capacity in Zea mays leaves. The method is fast and non-destructive so that high numbers of plants can be evaluated. Suitable models for prediction of leaf parameters from the leaf reflectance spectra were obtained using partial least square regression with recursive feature elimination. A minimum of about 50 samples from each species was required for reliable model development. In greenhouse material, the C/N ratio was the parameter resulting in the highest correlation between predictions and observations, but the final intra-species models could also predict photosynthetic parameters such as initial slope of the A-Ci curve and the maximal assimilation capacity with high accuracy. The obtained models will now be tested in leaf material from the field. Our results indicate that leaf reflectance phenotyping is an efficient method for improving crop photosynthetic capacity.

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P27

Regulatory networks and kernel length related genes identified by eQTL analysis in 5 DAP maize kernels

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Early maize kernel development produces most cells comprising the mature kernel and is thus closely related to final grain yield. However, the regulatory networks controlling gene expression during the early phases of maize kernel development remain unclear. In this study, by analyzing RNA sequencing data derived from 318 inbred lines, we identified 22,966 eQTLs regulating 18,377 expressed genes in 5 DAP maize kernels. Based on this, the regulator-target relationships between 4,408 trans-eQTLs and 4,980 genes were established by setting further criteria, in which putative molecular links of auxin, brassinosteriod, and/or cytokinin signaling pathways with cellular processes including cell cycle and division, cell differentiation and cell wall organization/ biogenesis were predicted. Furthermore, 137 genes that are expressed and regulated by eQTL at 5 DAP were revealed significantly associated with kernel length by an approach integrating eQTL with QTT analyses. These data lay the groundwork for fully understanding the molecular mechanisms underlying maize kernel development and provide candidate genes for molecular breeding.

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P28

Regulatory Networks Governing Nitrogen Use Efficiencyt in Maize

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Nitrogen (N) is an essential micronutrient for plants. Maximizing Nitrogen Use Efficiency (NUE) in plants is a critical way to increase crop production and reduce negative impacts on environment. In order to explore the gene regulatory network (GRN) that controls these processes, we have profiled the transcriptome of maize in response to N limitation. Based on this profile, we have used yeast one-hybrid (Y1H) technology to systematically map the gene regulatory network that governs the genes that are known to be involved in the process of nitrogen uptake, assimilation, utilization, remobilization and transcriptional regulation in maize. We have compared this regulatory network with an NUE network in Arabidopsis, conducted correlation analysis using expression data and identified key transcription factors that regulate maize genes involved in NUE.

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P29

Sequence analysis of european maize inbred line F2 provides new insights into molecular and chromosomal characteristics of presence/absence variants.

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Maize is well known for its exceptional structural diversity, including copy number variants (CNVs) and presence/absence variants (PAVs), and there is growing evidence for the role of structural variation in maize adaptation. While PAVs have been described in this important crop species, they have been only scarcely characterized at the sequence level and the extent of presence/absence variation and relative chromosomal landscape of inbred-specific regions remain to be elucidated.
De novo genome sequencing of the French F2 maize inbred line revealed 10,044 novel genomic regions larger than 1 kb, making up 88Mb of DNA, that are present in F2 but not in B73 (PAV). This set of maize PAV sequences allowed us to annotate PAV content and to analyze sequence breakpoints. Using PAV genotyping on a collection of 25 temperate lines, we also analyzed Linkage Disequilibrium in PAVs and flanking regions, and PAV frequencies within maize genetic groups.
We highlight the possible role of MMEJ-type double strand break repair in maize PAV formation and discover 395 new genes with transcriptional support. Pattern of linkage disequilibrium within PAVs strikingly differs from this of flanking regions and is in accordance with the intuition that PAVs may recombine less than other genomic regions. We show that most PAVs are ancient, while some are found only in European Flint material, thus pinpointing structural features that may be at the origin of adaptive traits involved in the success of this material. Characterization of such PAVs will provide useful material for further association genetic studies in European and temperate maize.

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P30

Sequence shattering and chromothripsis-like genome rearrangements following biolistic transformation in rice and maize

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We biolistically transformed linear 48 kb phage lambda molecules into rice and maize and analyzed the results by sequencing and optical mapping. Among a total of 8.2 Mb of inserted lambda analyzed, the average fragment size was ~200-500 bp. Fragments were joined by microhomology-mediated and non-homologous end joining, and organized in random arrangements. Optical mapping confirmed that one event contained a ~2.4 Mb insertion with a collage of thousands of small lambda fragments. Further analysis revealed similar rearrangements of the chromosomes in the transformed plants. There was clear evidence of large scale deletions, as well as degradation and reassembly of large chromosome fragments, including one entire rice chromosome. The structure of the inserts strongly resembles the outcome of chromothripsis, a chromosome fragmenting process frequently observed in late stage cancers. We suggest that particles frequently enter and exit nuclei to settle in the cytoplasm, where they form micronuclei that undergo degradation that occasionally rejoin with the nucleus.

Funding acknowledgement: National Science Foundation (NSF)

P31

Soil-based machine vision seedling emergence assay for studying cold tolerance in maize

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Seedling emergence is a critical stage in the establishment of a successful crop. Germination and robust seedling establishment are selected traits during the development of new varieties but with inefficient, largely manual methods. We developed an in-lab, soil-based machine vision platform to automatically measure emergence parameters, including rate, median time, duration, and percent. The assay is scalable, accommodates chemical or environmental treatments, and can be used with different soil types. A single camera monitors 168 kernels by time-lapse imaging. One current platform uses twelve cameras to monitor 2,016 kernels in parallel. A custom software tool employing machine learning processes the time-lapse images to determine the point of emergence. A public version of application runs on the CyVerse cyberinfrastructure. Maize seedling percent emergence is measured with a 2% False Negative Rate and median time to emergence is measured with a 30 minute temporal resolution. Cold tolerance of 40 diverse inbred lines was assayed by sowing and imbibing kernels in 5°C soil for 5 days. Flats were transferred to 24°C for the emergence assay. The treatment protocol gave a broad distribution of cold sensitivity. Total emergence after cold conditions ranged from 20% to over 100% of control conditions. Mean emergence time after cold treatment varied from 4 to 7 days after the shift to 24°C and mean emergence time did not correlate with percent emergence relative to control conditions. Several tropical lines were the most cold tolerant while some European and Midwestern dents were consistently cold sensitive suggesting that cold tolerance is not naturally selected by latitude and that tropical lines can be a source of cold hardy alleles. We are in the process of screening QTL mapping populations with parental inbred lines that have contrasting cold sensitivities.

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P32

Structural variation analysis in the Wisconsin Diversity Panel using multiple *de novo* genome assemblies

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Analysis of structural variation in maize has previously been limited by access to only a single reference genome assembly. With multiple *de novo* assemblies available within the species, we are able to expand our understanding of the extent of structural variation that exists within maize. To this end, we have sequenced the inbred lines in the Wisconsin Diversity (WiDiv) Panel to 10-40x sequence depth. Using a number of recently developed algorithms including LUMPY, MetaSV, and Genome STRiP, we have genotyped inbred lines from the WiDiv panel for structural variants relative to the B73, PH207, and W22 reference genome assemblies. Loci across the assemblies have been linked using a series of colinear chains. We are working towards using a machine learning approach to provide confidence to structural variation genotype scores across algorithms and assemblies. This information will ultimately be used to link genotypes and phenotypes in an association mapping framework to determine if structural variants preferentially affect certain traits and if the distribution of effect sizes differs between structural variants and more traditional, SNP-based effects.

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P33

The NUPRIME project: nuclease profiling of four reference tissues as a resource for maize epigenomics.

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The goal of this project (referred to as NUPRIME) is to develop micrococcal nuclease (MNase) profiling as a foundational resource for integration of maize epigenomic data. The web page at maizenucleosome.org describes the project, supported by the Plant Genome Research Program (NSF IOS 1444532). We previously showed that particular genomic regions are highly susceptible to variation introduced by differences in the extent to which chromatin is digested with MNase (Vera et al., 2014 Plant Cell). We exploited this digestion-linked variation to simultaneously map nucleosome occupancy and open chromatin, defining the functional portion of the maize genome (Rodgers-Melnick et al., 2016, PNAS). The open chromatin is nuclease hypersensitive and operationally defined by differential nuclease sensitivity (DNS). Here we present DNS-seq chromatin profiles on four distinct maize B73 tissues; root tip, coleoptilar nodes, earshoot, and mid-maturation endosperm. Two-week hands-on workshops have been conducted to train other scientists to apply DNS-seq chromatin profiling to their own samples, ranging from maize and sorghum to arabidopsis and pine. We also developed and applied a new peak-calling algorithm, iSeg (Girimurugan et al., BMC Bioinformatics, in revision, 2018), to identify discrete sites of open chromatin and facilitate comparative genomics. These data will be made and released through our UCSC maize genome browser, http://www.genomaize.org/.

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P34

The practical haplotype graph: using a simplified pan-genome to impute genotypes from skim sequence

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The Practical Haplotype Graph provides a general, graph-based, computational framework that can be used with a variety of sequencing methods to infer high-density genotypes directly from low-coverage sequence. The framework combines existing software with custom code implemented in a series of Docker modules that allows users to build custom analysis pipelines that can run on a variety of system architectures. The method first loads haplotypes from a population to a relational database. To genotype an individual, a graph is constructed from the haplotypes stored in the database. Sequence from the individual is then used with an HMM (hidden Markov model) to identify the most likely path through the graph. The resulting path is translated to variant calls and output in VCF format. By integrating an entire species worth of prior information, the PHG pipeline can produce an accurate whole genome sequence from any sequencing approach. Potential applications range from basic genomic research into chromatin structure to applied plant breeding.

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P35

Tracing the heritability of agronomic traits in maize

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Remarkable morphological changes were accumulated along the modern breeding process of maize (Zea mays ssp. mays). While, a comprehensive dissection of the underlying genetic architecture is challenging. To this end, we resequenced 775 temperate maize breeding accessions and constructed a high-density map of ~63.60 million SNPs. Purifying selection was detected to keep the deleterious mutations towards low frequency during modern breeding. Despite the fact that only 9 of 20 agronomic traits have significantly associated loci ($P < 6.3 \times 10\text{-}8$), genome-wide SNP based heritability (h2 SNP) was estimated to be as high as ~0.77 (an average of ~0.37), indicating that SNPs with small effects may jointly contribute to the "heritability". By partitioning the h2 SNP into SNPs with different allele frequency spectrums, we found the low frequency (MAF < 0.01) alleles, which were rarely analyzed in traditional association studies due to limited sample size and genotyping efforts, could contribute as much as ~17.1% of phenotypic variance. Our findings provide novel insights into the genetic architecture of complex agronomic traits and will benefit the design of molecular breeding and genome selection (GS) in maize.

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P36

Tripsacum de novo transcriptome assemblies reveal parallel gene evolution in maize and *Tripsacum* after ancient polyploidy

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Following a whole genome duplication event, plant genomes reduce in size. When there isn't selective pressure to maintain duplicate gene copies, one copy is lost. Maize and its sister genus, *Tripsacum*, share a genome duplication event that occured 5-26 million years ago. It is unknown whether *Tripsacum* grasses and maize have maintained a similar set of genes under purifying selection because few genomic resources for *Tripsacum* exist. Here we present high quality de novo transcriptome assemblies for two species: *Tripsacum dactyloides* and *Tripsacum floridanum*. The transcriptome assemblies have L50 lengths of 1,442 bp and 1,452 bp in *Tripsacum dactyloides* and *Tripsacum floridanum*, respectively. Core plant eukaryotic genes are well represented because 84% and 86% of BUSCO genes are completely assembled in *Tripsacum dactyloides* and *Tripsacum floridanum*, respectively. A previous proteomics study by Walley et al. (2016) identified protein-encoding maize genes by analyzing 33 tissues. We hypothesized that these protein-encoding genes are resisting fractionation in both maize and *Tripsacum*, and that the remaining genes in the genome are more likely to decay into pseudogenes. Protein-encoding maize transcripts and their *Tripsacum* homologs have higher GC content, higher gene expression levels, and more conserved expression levels than putatively untranslated maize transcripts and their *Tripsacum* homologs. These results indicate that genome fractionation is occuring in a similar fashion in two genera after a shared ancient polyploidy event. The *Tripsacum* transcriptome assemblies provide a high-quality genetic resource that can frame the maize genome in a larger evolutionary context.

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

P37

Using two parent genome mapping to identify expression level quantitative loci in maize roots

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Roots of young plants undergo highly regulated cell differentiation that patterns root architecture and physiology and has lifelong effects on the structural integrity, water-use efficiency, and nutrient flow of the plant. To understand how gene expression in maize roots impacts different aspects of plant structure and function, we measured transcript levels in two-week-old roots of 218 greenhouse-grown plants belonging to the maize Intermated B73 x Mo17 (IBM) recombinant inbred population. After performing quality control steps, we retained an average of 19.6 million reads per sample. The high levels of sequence diversity in maize can cause artifacts in alignment-based methods of expression quantification, especially if one of the parents is the reference sequence. If RILs that contain the B73 allele at a particular locus have an inflated expression level of that transcript due to alignment differences, expression QTL (eQTL) mapping may detect a false positive association between the B73 allele and expression of a transcript. To date, there has been no well-tested and standardized method for dealing with mapping bias in a bi-parental RIL population. We have devised an approach utilizing the reference genomes of each parent to account for mapping bias. By using reference genomes from both B73 and Mo17 for eQTL mapping, as well as the difference in expression values between references, we can detect instances of false positive eQTL that arise from reference genome discrepancies including variation in paralogs between references. The results of parent-sample alignments to the congenic and dysgenic references can identify loci likely to encode cis-eQTL arising from alignment bias and help to validate cis-eQTL driven by true expression differences. After using both references to confirm a high-confidence set of eQTL, we intend to perform network analyses and gene ontology enrichment tests to identify functional gene regulatory modules in the developing maize root.

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P38

A system biology approach to identify biochemical markers representative of high yielding maize lines

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We have developed a systems biology approach in order to identify metabolic pathways that could play a major role in the control of grain production in maize. Using nineteen genetically distant maize (Zea mays L.) lines from Europe and America, metabolomic, biochemical, fluxomic, and metabolic modelling approaches were combined in order to identify key metabolic and enzymatic markers representative of high yielding lines. Both correlation studies and metabolic network analyses allowed the description of a maize ideotype with a high grain yield potential. Such an ideotype is characterized by low accumulation of soluble amino acids and carbohydrates in the leaves and high activity of enzymes involved in the C4 photosynthetic pathway and in the biosynthesis of amino acids derived from glutamate. Chlorogenates appear to be important markers that can be used to select for maize lines that produce larger kernels. We will discuss how these markers could be further used for breeding and agronomic purposes under high and low fertilization input, using genetic engineering, mutagenesis and association genetics.

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P39

An opaque phenotype and altered mitochondrial respiratory chain are caused by a maize *rug3* mutation

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The importance of C partitioning and C/N balance in kernels led to a focus on genes that could affect these features. An "opaque" phenotype, for example, is indicative of defects in the protein-rich vitreous layer. Here, a new opaque mutant was identified from the UniformMu maize population and confirmed by transgenic complementation to arise from disruption of RUG3 (RCC1/UVR8/GEF-like3). The *rug3* mutant kernel had a normal starchy endosperm, but a defective vitreous layer. Protein profiles showed that the proportion of zeins was significantly decreased. The visible kernel phenotype was severe when grown in spring, but less altered during the autumn planting season in Florida. Analysis of transcripts showed deficient splicing of mitochondrial (mt) mRNAs, including those for complex I of the respiratory chain. RUG3, which belongs to the RCC1 superfamily, promotes folding and subsequent splicing of group II introns from mt genes. The RUG3 splicing factor functions with other diverse, nuclear-encoded splicing factors that are targeted to mitochondria. These other factors include PPR proteins (Pentatricopeptide Repeat), and maturases. Although RCC1 proteins have similar structures to one another, their functions are quite diverse, ranging from guanine nucleotide exchange for Ran GTPase, to UV-B light signaling, and mt mRNA splicing. Maize RUG3 mediates splicing of the mt gene, *nad2*, which encodes a subunit of complex I. Although plants have evolved alternative pathways for electron transfer, ATP production relies heavily on complex I. Disruption of complex I and its specific effect on respiration and other mitochondrial functions thus affects the development of kernels. Transcriptomic profiles suggested that mutants had lower levels of mRNAs for biosynthesis of both starch and storage proteins, but also increased abundance of transcripts for constituents of the entire respiratory chain. The latter might possibly result from a compensation effect mediated by ROS or an energy-sensing system.

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P40

Apoplasmic phloem loading in *Zea mays L.* requires three SWEET sucrose transporters

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Crop yield depends on efficient allocation of sucrose from leaves to seeds. Sugar is transported from the leaves where it is synthesized, to phloem for long distance transport, to sink tissue such as seeds and roots. In Arabidopsis, phloem loading is mediated by a combination of SWEET sucrose effluxers and subsequent uptake by SUT1/SUC2 sucrose/H+ symporters. However, it is not clear that this is true for monocots: rice does not appear to require SUT-medated apoplasmic transport for phloem loading, whereas maize does require SUT1. We analysed the contribution of SWEETs to phloem loading in maize. Three leaf-expressed SWEET sucrose transporters, *ZmSWEET13* paralogues a, b, and c, are among the most highly expressed genes in the leaf vasculature. Genome-edited triple knock-out mutants were severely stunted. Photosynthesis in mutant plants was impaired and leaves accumulated high levels of soluble sugars and starch. RNA-seq revealed profound transcriptional deregulation of genes associated with photosynthesis and carbohydrate metabolism. GWAS analyses may indicate that variability in *ZmSWEET13s* correlates with agronomical traits, specifically flowering time and leaf angle. This work provides support for cooperation of three ZmSWEET13s with ZmSUT1 in phloem loading in *Zea mays L.*, and suggests a potential target for improving photosynthesis and sugar partitioning efficiency.

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P41

barren stalk3 is required for axillary branch development and maps to the same location as *barren stalk2*.

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Zea mays (maize) bears female reproductive inflorescences, ears, on axillary branches. Both initiation and maintenance of the female axillary meristems are necessary for their proper development. Previously characterized *barren stalk* (*ba*) mutants have determined that auxin is required for initiation of these axillary branches. The *ba* mutants, *ba1* and *ba2*, encode a transcription factor and interacting protein, respectively, that function downstream of auxin to control development of axillary meristems. A new mutant, *barren stalk3* (*ba3*), was identified in 1990 in an *Ubiquitous* transposon active population by Pan and Peterson as a novel locus controlling this process. The *ba3* mutant failed to initiate an axillary ear branch and the grooves on the stem that normally bear ears do not develop. In the B73 genetic background, *ba3* mutants have shorter tassel branches and fewer secondary branches but there was no effect on plant height or tassel length. An enhancer of the *ba3* mutant phenotype was discovered when introgressed into the Mo17 background. The *ba3* mutants were significantly shorter for plant height and tassel length and there were fewer primary and secondary tassel branches on *ba3* mutants as compared to normal siblings. Therefore, the *ba3* locus was necessary for ear initiation without affecting plant height and the mutant phenotype was enhanced by maize standing variation and we infer that the *ba3* gene is necessary for axillary meristem initiation or maintenance. We used a next generation sequencing and bulk-segregant analysis approach to map the *ba3* locus to the short arm of chromosome 2, indicating that it could, in fact, be a new allele of *ba2*. Current endeavors are underway to identify the causative mutation of the *ba3* phenotype and confirm if it is an allele of *ba2*. These results indicate that there may only be two identified barren stalk loci in maize.

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P42

Biochemical and structural characterization of a new maize receptor-like kinase likely involved in drought stress.

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Protein kinases and receptor-like kinases (RLKs) are key regulators of virtually every physiological process. However, despite their importance, most plant kinases are "under-explored". By analyzing the gene expression profile of maize plants subjected to drought stress we have selected some promising targets including a gene that encodes a leucine-rich repeat receptor-like kinase (LRR-RLK). Here we describe the cloning, expression, purification and crystallographic structure of the kinase domain of Zm00001d028770. Small molecules inhibitors were identified to be used chemical biology studies towards the understanding of the mechanism of action of this LRR-RLK protein in plant response to drought stress. ITC analysis confirmed the kinase inhibitor ENMD-2076 as a ligand to kinase domain with high affinity. This maize LRR-RLK seems not have kinase activity as neither ATP nor GTP binds its kinase domain. We are using CRISPR/Cas9 genome editing to produce loss of function mutants to understand the function of this LRR-RLK kinase better. A CRISPR/Cas9 construct was generated expressing Cas9 under control of the ZmUBI promoter and two sgRNAs targeting Zm00001d028770 expressed by OsU6-2 pol III promoters. The primary focus of this work is to develop tools to establish a chemical biology platform to study the maize kinase set potentially involved in drought stress response.

Gene / Gene Models described: Zm00001d028770
Funding acknowledgement: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP)

P43

Characterization and functional analysis of maize Terpene synthetase6 (TPS6) Gene

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Maize plants emit a complex blend of secondary metabolites to defend the biotic stress and abiotic stress. Terpene synthetases were key enzymes catalyzing secondary metabolite terpenes biosynthesis. TPS6 gene encoded a sesquiterpene synthetase, which catalyzed β-macrocarpene and β-bisabolene. In prior studies, TPS6 expression was induced by bacteria and pest damage, indicating that TPS6 gene may be involved in the process of disease and insect defense. In our studies, no TPS6 gene expression was detected in B73 maize leaf and root with JA or SA induction and TPS6 gene mainly expressed in maize root not leaf induced by ABA hormone. To further elucidate the TPS6 gene's transcriptional regulation, the different truncated TPS6 promoter's activity were analyzed in transgenic *Arabidopsis thaliana* and the result showed -400 bp (ATG as +1) promoter included the sufficient *cis*-elements and had the same activity as the full length promoter (1500 bp). A high-throughput screening of an *Arabidopsis* transcription factor library using 400 bp promoter region as the bait identified a DREB transcription factor as candidate regulator. Within the 400 bp promoter region, we found an 8 nt *cis*-element was necessary to gene expression. Ninety-six maize inbred lines were re-sequencing and 44 inbred lines missed the 8 nt sequence which resulted in no TPS6 gene expression in maize root induced by ABA. The other 52 inbred lines including 8 nt sequences in their promoters could induce TPS6 gene expression in root. The role of 8 nt *cis*-element to TPS6 expression was ongoing study. The transgenic maize plants with TPS6 gene overexpression and CRISPR-Cas9 knock-out were obtained. GC-MS showed the release of β-macrocarpene and β-bisabolene in OX-transgenic maize root and leaf. The further works focus on the TPS6 transcriptional regulation mechanism and functions in disease, pest, and drought defense.

Gene / Gene Models described: *TPS6*; GRMZM2G127087
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P44

Cloning and characterization of a gene which disrupts carbohydrate partitioning in maize

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Carbohydrate partitioning is the process through which plants distribute photoassimilated carbon to distal growing and storage tissues. The physiological aspects governing this process are well characterized; however, the underlying genetic mechanisms are poorly understood. To gain better insight into the genes controlling carbohydrate partitioning, we screened an EMS-mutagenized population for plants that displayed phenotypes characteristic of the inability to effectively transport carbon, such as reduced plant height, leaf chlorosis, and the hyperaccumulation of starch in the leaf. Through this screen we identified many mutant plants displaying these phenotypes, including a mutant identified as *carbohydrate partitioning defective6 (cpd6)*. An additional mutant, *cpd84*, displayed strikingly similar phenotypes to *cpd6*, and, through complementation testing, was identified as being allelic. In addition to the above phenotypes, *cpd6* mutant leaves display a reduction in photosynthesis and have increased levels of sugars and starch. The mutation responsible for the *cpd6/84* phenotype was identified through genetic fine mapping. Sequencing of the candidate gene revealed that each mutant allele was caused by a premature stop codon, with the position of the *cpd84* mutation being downstream of the *cpd6* mutation. We are currently conducting experiments to identify the cell-type specific expression, subcellular localization of the encoded CPD6 protein, and determine its activity. Through identifying *Cpd6* and similarly functioning genes we are increasing our knowledge of the regulation of carbohydrate partitioning, which will contribute to the development of crops with improved yields and stress tolerance.

Funding acknowledgement: National Science Foundation (NSF)

P45

Development of an amenable system for site-specific addition to a maize B-Chromosome

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Currently, transgenic crops are created by random integration of a gene into the crop plant. This works for single gene traits, such as herbicide resistance, but not as well for complex traits, which require multiple genes to be expressed. For multiple transgenes to be expressed in a crop plant, several genes are randomly integrated into the plant. Identifying plants that have all the separate transgenes integrated becomes a very difficult task. Gene stacking at a single location in the genome would make combining multiple transgenes into plants a simpler process. My presentation focuses on the development of a system that allows for transgenes to be sequentially added to a specific site in the maize genome. The system would work by utilizing two recombinases, Cre recombinase and ϕC31 Integrase, to remove a selectable marker and to integrate transgenes. An initial construct containing a selectable marker, flanked by loxP sites, which are acted upon by Cre recombinase, and an attP site, is transformed into a maize plant. The selectable marker is then removed from the integrated transgene by exposure to Cre recombinase. Two amendment constructs would enable modification of the integrated construct by utilizing complementary attP and attB sites, which are acted upon by ϕC31 Integrase. The amendment constructs contain cargo and a promoterless selectable marker which, upon successful recombination with the initial site, would restore expression of the selectable marker. Successful demonstration of this system would simplify inclusion of multiple transgenes, and the synthesis of multi-gene pathways in plants.

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P46

Effect of sugar levels on the low oxygen response of maize roots

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Hypoxia (limited oxygen availability) typically leads to energy imbalances with deleterious effects at cellular-to whole-plant levels. Sugar availability is central to hypoxia tolerance because fermentable substrates are essential for recycling ATP. In maize, early work indicated sugar and oxygen modulate accumulation of mRNAs encoding a small group of anaerobic proteins (ANPs). The objective of work presented here was to identify genome-wide effects of sugar levels on the hypoxic response of maize. Toward this end, root tips were cultured under aerobic (20% O_2) or hypoxic (0.2% O_2) conditions with either physiologically abundant glucose (2%) or limited glucose (0.2%). Transcriptome responses were quantified throughout treatments by RNA-seq. The combination of sugar and oxygen levels tested here led to major transcriptome restructuring within 3h. The response was consistent with altered metabolic profiles observed within the same time-frame. Sugar- and oxygen-responsive mRNAs were examined separately. The number of sugar-responsive genes was constricted by hypoxia while low glucose shifted the profile of low-oxygen responsive genes, possibly due to starvation. Over-represented transcripts were mostly associated with stress tolerance and survival including those for carbohydrate metabolism and glycolysis. Regardless of oxygen levels, physiologically abundant glucose had a significant effect on transcripts associated with RNA processing and protein synthesis. Co-expression network analysis identified genes with similar expression patterns across different sugar levels. Low glucose limited the number of co-expression relationships of ANP transcripts indicating that the negative effects of hypoxia on root transcriptome are exacerbated by starvation. Changes in transcriptional relationships throughout the treatments were consistent with coordination of transcriptome restructuring and changes in sugar status. Results presented here show novel contributions of sugars to co-expression relationships among transcripts under low oxygen conditions and the potential contributions to their modulation.

Funding acknowledgement: National Science Foundation (NSF)

P47

Effect of water-deficit on phenotypes and transcriptomes of developing tassel in maize

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Maize often exhibits asynchronous pollination under abiotic and biotic stress conditions, but the molecular basis of asynchronous pollination has not been elucidated. Tassel development is a key process which affects the anthesis-silking interval(ASI). In this study, we observed significantly increased ASI in the inbred lines of B73 and Chang7-2 under water deficit, and both inbreds displayed delaying pollen shedding and longer barren tip length with decreased yields. Our transcriptome analysis identified 1931and 1713 differentially expressed genes (DEGs) in immature tassels of B73 and Chang7-2 between well-watered and water deficit. We also identified 28 transcription factors from co-DEGs of two inbreds, some of which were known to regulate development, flowering and stress processes. Collectively, we demonstrate a molecular association of the regulations of tassel development with water deficit stress at the early vegetative stage. This finding extends our understanding to the molecular basis of maize tassel development under drought stress.

P48

Elucidating the transcriptional regulatory network controlling SUT and SWEET genes in maize

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Carbohydrate partitioning describes the process of how sugars (typically sucrose) produced during photosynthesis are distributed from source organs (primarily leaves) to sink organs (e.g., roots, stems, flowers, and fruits) where these sugars are catabolized, anabolized, or stored. In maize, phloem loading in source organs requires sucrose crossing the apoplasm (cell wall space) between the mesophyll and companion cells in leaves. In many sink tissues, such as kernels, phloem unloading likewise requires sucrose to transit the apoplasm between companion cells and parenchyma cells. The coupled activities of two families of transporters—one that imports sucrose and one that exports—underlie sucrose movement across membranes. The Sucrose Transporter (SUT) gene family encodes sugar-proton symporters, most of which import carbohydrates from the apoplasm. SUTs work in conjunction with the SWEET transporters (Sucrose Will Eventually be Exported Transporters), which function as passive efflux proteins. To elucidate the regulatory network that governs SUT and SWEET genes expression, we are using a yeast one hybrid screen to identify transcription factors that control their expression. To this end we have cloned SUT and clade III SWEET promoters into yeast expression vectors and begun screening a library consisting of 2200 maize transcription factors (Burdo et al., 2014, Plant J 80:356). Here we present the results of our initial screening efforts and current understanding of SWEET and SUT gene regulation.

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P49

Enhancement of maize disease resistance by CRISPR/Cas9 editing of LOX and WRKY genes

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Fusarium verticillioides (Fv) causes ear rot in maize and contaminates the kernels with fumonisins, a family of mycotoxins that affects feed and food and considered carcinogenic for humans and animals. Several studies were conducted to identify maize genes associated with host plant resistance to *Fv* infection and fumonisin accumulation. It is known that plant lipoxygenase (LOX)-derived oxylipins regulate defense against pathogens and that the host-pathogen lipid cross-talk influences the pathogenesis.
The genome editing technology of Clustered Regularly Interspaced Short Palindromic Repeat/associated Cas9 (CRISPR/Cas9) was applied in order to investigate the possible implication of the lipoxygenase gene *ZmLOX6* and the transcription factor *ZmWRKY125* in the resistance mechanisms against *Fv*. The enhanced expression of these genes was previously observed by RNA-seq experiments in maize resistant genotypes and GWAS resulted in a SNP significantly associated with *ZmWRKY125*.
The CRISPR cloning was based on a double cloning using two different guides (sgRNA) for one gene target. The constructs under the maize promoter *ZmpUBI* in the binary vector p1609 were transformed into the maize A188 line using *Agrobacterium tumefaciens* mediated transformation. Edited *ZmLOX6* and *ZmWRKY125*-expressing maize plants will be characterized for *Fv* resistance using *in vivo* assays.
In parallel, maize mutants carrying Mu insertions in the *ZmLOX4* gene were screened for *Fv* resistance and the expression profiles of 15 genes of the LOX pathway were studied. The fumonisin content and the enzymatic lipoxygenase activity will be also investigated in the *lox4* mutant kernels.

Gene / Gene Models described: *GRMZM2G109130;GRMZM2G109056;GRMZM2G102760;GRMZM2G040095; GRMZM2G104843;GRMZM2G015419;GRMZM2G009479;*;
Zm00001d033623;Zm00001d033624;Zm00001d013493;Zm00001d002000;Zm00004b024196;Zm00001d015852;

P50

Exploration of the molecular mechanism of CMS-S

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Two CMS-S specific chimeric open reading frames, orf355 and orf77, were considered as CMS candidate genes, the amounts of its 1.6-kb transcripts were previously shown to be greatly reduced in fertility-restored microspores relative to the amounts in sterile plants. However, little is known regarding its mechanism of action. In this study, we found that transgenic plants(Maize and Arabidopsis) containing MTS-orf355-GFP construct exhibiting typical gametophytic male sterility, which comfirmed that orf355 was the CMS gene. The mitochondrial OXPHOS activity was obviously impaired at microspore stage in CMS-S tassel, from which the CMS-S microspore start to collapse. Retrograde reponse was activated in CMS-S tassel compared with fertile ones. We found that the NAD/NADH redox state ratio reduced in both tassel and ear mitochondrial at microspore stage,while further reduced in tassel but keep constant in ear. This indicate exsiting of a tassel specific regulator mediate the collapsion of mircrospore. By yeast-one-hybrid screening, we identified an tassel specific transcription factor named DBP4, which may participate in this process. DBP4 binding to orf355 promotor with the DRE/CRT element. The expression level of DBP4 increase dramaticaly at microspore stage in CMS-S tassel, but not change in control fertile tassel. DBP4 was an stress responsive transcription factor, analysis its promotor identified several typical cis UPR(unfolded protein response) element. Overexpression of DBP4 in Arabidopsis resulting in gametophatic male sterility.

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P51

Functional characterization of maize auxin signaling modules in yeast

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The hormone auxin regulates myriad processes during the life of a plant - from root and shoot development to environmental responses. Understanding how auxin regulates such diverse processes necessitates characterization of the specific signaling modules (receptors, repressors, transcription factors) that enable plant cells to detect and respond to auxin. Recapitulation of the Arabidopsis nuclear auxin signaling system in yeast has shown that auxin repressors (Aux/IAAs) exhibit a range of auxin-induced degradation rates which can be tuned depending on identity of the co-expressed auxin receptor and of specific amino acid sequences within the repressors. Subsequent studies confirmed that Aux/IAAs show similar degradation differences *in planta* and that Aux/IAA degradation dynamics are highly correlated with the rate of developmental events. We are now using this yeast system to functionally annotate auxin signaling modules crucial during maize reproductive organogenesis. We have identified the subset of maize Aux/IAAs expressed in developing inflorescences and have utilized the yeast system to confirm that these repressors degrade in response to auxin and exhibit different degrees of hormone sensitivity. Efforts are now centered on characterizing transcriptional repression across this subset of maize Aux/IAAs. This work is providing new insights to inform our understanding of how auxin action is specified by context-specific deployment of auxin signaling components during plant development.

Gene / Gene Models described: *ZmIAA1, ZmIAA2, ZmIAA4, ZmIAA5, ZmIAA8, ZmIAA9, ZmIAA10, ZmIAA12, ZmIAA14, ZmIAA16, ZmIAA20, ZmIAA21, ZmIAA25, ZmIAA27, ZmIAA28, ZmIAA29*; GRMZM2G079957, GRMZM2G159285, GRMZM2G104176, GRMZM2G004696, GRMZM2G167794, GRMZM2G057067, GRMZM2G037368, GRMZM2G142768, GRMZM2G077356, GRMZM2G121309, GRMZM5G864847, GRMZM2G147243, GRMZM2G115357, GRMZM2G130953, GRMZM2G035465, GRMZM2G163848
Funding acknowledgement: National Science Foundation (NSF), MJ Murdock Charitable Trust, Whitman College

P52

GBS identification of genomic loci associated with embryo twins in *ig1* mutants

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Maize embryo twins occur rarely, in fewer than 0.1% of ovules in most maize lines. The INDETERMINATE GAMETOPHYTE1 (*IG1*) gene encodes a protein required for regulation of embryo sac development; when this gene is mutated, the occurrence of embryo twins increases dramatically, depending on the genotype, reaching as high as 11% of Mo17 ovules.
B73 *ig1* plants have a low embryo twin rate, and hybrid B73/Mo17 *ig1* plants display an intermediate rate, suggesting a single genetic modifier present in Mo17. We employed a transgenic Mo17 line carrying an *ig1-O* W23 segment on chromosome 3 to produce a mapping population in which one chromosome is pure Mo17 and the other carries the *ig1* locus on a 50% B73, 49% Mo17+1% other background. Seeds from this population were sorted into groups containing twin and non-twin embryos, and checked via PCR for the presence of the *ig1* locus. Embryo tissue was then sequenced and mapped to the B73v4 genome using a GBS protocol. The resulting maps, from 48 twin and 45 non-twin samples, reveal two strongly segregated loci, on chromosomes 8 and 9. Several SSR markers display significant segregation in nearby regions. The B73 Chr8 locus maps to Chr8 on Mo17 (v1) while the B73 Chr9 locus maps to Chr5 on Mo17. We therefore posit that Mo17 contains a genetic modifier which promotes embryo twin production in *ig1* mutants on its chromosomes 5 and 8.

Funding acknowledgement: National Science Foundation (NSF)

P53

Genetic variability for nitrate uptake in a core collection of European and American maize (Zea mays L.) lines.

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Nitrogen use efficiency (NUE) in crops can be defined as the grain yield per unit of available nitrogen (N) already present in the soil and added as N fertilizer. Thus, improving NUE in crops is a way of reducing both the cost and the detrimental environmental effects associated with N fertilization. NUE is the product of N uptake efficiency and N utilization efficiency. At high N input, variation in NUE was explained by variation in N uptake capabilities. Generally, cereals such as maize are inefficient at acquiring N from the soil. Thus, identifying genotypes that are more efficient in capturing mineral N resources and identifying both the phenotypic traits and the biological mechanisms controlling the ability of maize to take up N is of major importance. To explore N uptake efficiency, a core collection of nineteen maize inbred lines originating from different countries of Europe and America, was grown in hydroponics under non-limiting N supply. When the plants had 4 visible leaves, (nitrate) NO3- uptake was measured using 15N labeled NO3-. In parallel, the architecture of the root system including seminal and nodal roots was analyzed using the WinRhizo software and the accumulation of mRNAs encoding NO3- transporters was measured using Quantitative Real-Time (Q-RT)-PCR. We found that within this natural population of maize lines, there is a very large variability for NO3- uptake efficiency, which is not necessarily correlated with the architecture of the roots and the accumulation of mRNAs encoding high and low affinity NO3- transporters.

Funding acknowledgement: Institut National de la Recherche Agronomique (INRA)

P54

Genome imbalance impacts global gene expression and small RNA expression in maize

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It has been long known that genome imbalance caused by changing the dosage of individual chromosomes (aneuploidy) has a more detrimental effect phenotypically than by varying the dosage of complete sets of chromosomes (polyploidy). An explanation of this phenomenon could be that genes with varied copy number exhibited a dosage effect. Later on, several studies illustrated that those dosage-sensitive modifiers are likely to be transcription factors and signaling components, which usually compose protein complexes. A gene balance hypothesis attempting to address the molecular basis for genome imbalance demonstrates that altering the stoichiometry of members of oligomeric complex will affect the function of the complex as a whole. If the complex affected plays a critical regulatory role in gene expression, it would consequently alter global gene expression. Despite all these findings, the mechanism of genome imbalance is still unclear. In order to investigate how genome imbalance affects gene expression, we performed an RNA-seq study of maize leaf tissue from aneuploidy and polyploidy series. Ratio distributions of experimental and control reads were generated to examine the trend of gene expression in aneuploidy or polyploidy in comparison to the diploid control. The result indicates genes both on the varied chromosome and the remainder of the genome show significant change of expression. In general, we observed a greater spread of modulation in aneuploids than polyploids. Recently, non-coding RNAs are discovered to play a key role in the regulation of gene expression. To understand whether non-coding RNAs are involved in genome imbalance, we analyzed the expression of small RNAs (20-24nt) in aneuploids and polyploids. This study will gain insight into the mechanism of genome balance and guide us to understand gene regulatory networks and genome function in maize.

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P55

Genomic analyses of the genetic basis for shaping maize plant architecture for adapting to increased planting density

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Increasing planting density has been a primary factor for the increased maize grain yield in the past few decades. High-density tolerant ideal plant architecture can optimize canopy architecture, improve photosynthetic efficiency, and prevent lodging, thus resulting in overall high grain yield. The complexity of maize genome has impeded the identification and functional studies of key genes controlling plant architecture in maize. Recently, we showed that in Arabidopsis, phytochorme B (phyB) and PIFs-mediated light signaling pathway interacts the miR156/SPL pathway to regulate different aspects of plant architecture (including leaf production, stem elongation, branching and flowering) in response to shade or high density planting. To investigate whether this genetic pathway also operates in maize, we conducted a systematic study of the maize PIFs and SPL gene family using the CRISPR-cas9 knockout and overexpression strategies. Progress on functional characterization of maize PIFs and SPLs in regulating various aspects of plant architecture will be presented.
In a further effort to identify the key regulatory genes and genetic pathways regulating plant architecture in maize, we collected 350 maize inbred lines (US-public and EX-PVP lines, as well as breeding lines used in China from 1960s to 2010s) and conducted multi-year and multi-location field experiments. Remarkable morphological changes were detected in plant architecture associated with high density planting during maize breeding, including early flowering, reduced ear height, more upright leaf angle, reduced tassel branches number, and shortened anthesis-silking interval. We also resequenced these 350 lines (more than 10X depth for each line) and obtained ~25 millions high quality SNPs. GWAS analysis allowed us to confirm several known genes and dozens of novel candidate genes that may have played pivotal roles in shaping maize architecture for adapting to high density planting.

Funding acknowledgement: National Science Foundation of China (NSFC)

P56

Heritable differences in C4 photosynthetic sub-type across diverse maize germplasm indicate utility in discover and breeding

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Maize, Sorghum, Sugarcane, and other important crop species rely on C4 photosynthesis for carbon fixation and growth. The current dogma is that C4 photosynthesis in maize is done primarily through the efficient NADP-ME C4 sub-type pathway. However, work stretching back over a decade indicates that in conjunction with NADP-ME, maize also utilizes the PCK sub-type of C4 photosynthesis, which is estimated to contribute up to 25% of overall photosynthetic product. This mixture of sub-types is hypothesized to enhance maize productivity and/or enable greater environmental flexibility. Still, the outstanding question remains: How much, if any, of the ratio between NADP-ME and PCK in maize is determined by genotype, and how much is determined by environment? In other words, is the C4 sub-type ratio in maize heritable, variable, and potentially useful in breeding?
RNAseq data from across the Maize 282 association panel and NAM founder lines was used to demonstrate that NADP-ME:PCK ratios in diverse inbred lines of maize vary considerably. These expression data were validated through in situ hybridization on mature maize leaves from representative inbred lines, as well as a replicated growth chamber experiment in controlled high- and low-light intensities. Quantitative genetic modeling of the NADP-ME:PCK ratio reveals a heritability of 0.54, indicating the potential utility of this trait in variant discovery, genomic selection, and maize breeding.

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

P57

High resolution interactomics in the maize leaf growth zone, using AN3 as a case-study

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Plant organ growth is driven by coordinated cell proliferation and expansion. The maize leaf is an excellent model system to study the complex regulation between these two developmental processes because it has a large growth zone in which dividing and expanding cells are organized in a linear fashion. This allows for high resolution sampling of dividing and expanding cells while still maintaining sufficient input for genome-wide molecular techniques. Combining such a sampling strategy with affinity purification techniques such as AP-MS and ChIP-seq allows the construction of dynamic interaction networks surrounding known growth regulators.
Such a dynamic network was generated in our lab surrounding ANGUSTIFOLIA3 (AN3), also called GRF-interacting Factor1 (GIF1), a transcriptional coactivator involved in leaf development. Tandem Affinity Purification (TAP-MS) showed that AN3 stably interacts with a SWI/SNF chromatin remodeling complex throughout the growth zone but interacts differentially with multiple GRF (Growth Regulating Factor) transcription factors depending on the developmental context. Quantification of these differential interactions shows enrichment of several GRFs in the division zone, GRF1 being the most prominent, while GRF4 and GRF10 interact stably throughout the growth zone. By performing the reverse TAP-MS using GRF1 and GRF10 as bait proteins, we show that GRF1 binds specifically to AN3/GIF1, while GRF10 binds specifically to the other two members of the GIF family, GIF2 and GIF3. Strikingly, TAP-MS using the GRF proteins as bait failed to identify any SWI/SNF complex components, hinting that AN3 might bind to the SWI/SNF complex and the GRF proteins independently, comprising two discrete types of proteins complexes.
Our current research focusses on extending this growth network to include the target genes of AN3, GRF1 and GRF10 by using ChIP-seq and to overlay the different types of interactomics data to understand how the network around AN3 and GRFs changes throughout the maize leaf growth zone.

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P58

INDETERMINATE1 direct targets and regulatory mechanisms

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INDETERMINATE1 (ID1) protein is a zinc-finger transcription factor that acts as a key regulator of the autonomous flowering pathway in maize. ID1 regulates the expression of florigenic genes, such as *Zea mays CENTRORADIALIS8* (*ZCN8*) in mature leaf. ID1 also controls different aspects of carbohydrate metabolism, establishing a physiological energy state in the plant that promotes the transition to flowering. Previously, RNA sequencing transcriptome analysis of wild-type and null id1 mutant plants was conducted to find ID1 targets in immature and mature leaf. Since ID1 is expressed exclusively in immature leaf, ID1 must act indirectly to regulate expression of its mature leaf targets. For example, evidence suggests that ID1 is required to establish a specific histone modification pattern in *ZCN8* chromatin, which is maintained throughout leaf development and facilitates florigen expression in mature leaf.
The goal of the present study is to identify ID1 direct targets (i.e. sequences directly bound by ID1) at a genome-wide level. To this end, we employed ChIP-seq and DAP-seq approaches in combination with previous RNA-seq data. This was followed by ChIP-seq to analyze alterations in histone modifications at loci bound by ID1, to assess whether the establishment of specific histone modification patterns is a common feature of ID1 regulatory mechanisms.
Our work has produced a list of putative ID1 direct targets, containing different transcription factors, which provide information to analyze mechanisms within the intricate regulatory network, involving ID1 control of flowering. These findings will be illustrated in the present poster, along with data describing the connection of ID1 with histone modifications of its targets.

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P59

Investigating seed mineral composition of Korean landrace corns (Zea Mays L.)

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Maize kernel is one of the greatest agricultural products in the world and used for various purposes as food, feed, and industrial materials. Manipulating the chemical composition of maize seeds has great impacts on its nutritional and industrial value. Maize is also a good target for mineral biofortification to solve the mineral malnutrition problems from which over 2 billion people are suffered. In this study, we collected maize germplasms including Korean landraces and quantified the amount of minerals in whole kernels and detected relationships among amount of minerals and seed phenotypes including texture and color. Twelve minerals were quantified by ICP-AES from 47 maize germplasms including 25 Korean landraces, 7 Korean cultivars or inbred lines, 4 inbred lines of USA, and 11 landraces from other countries. The amounts of Fe and S are significantly different among the groups with different seed colors. And the amounts of K, P, and S showed possible relationships to seed texture phenotype. Strong positive relationships were detected between the amount of P and those of K, Mg, and Mn, respectively. Between the amounts of Mn and Mg, a strong positive relationship was also detected. These results provide information about Korean corn landraces which was not intensively studied so far. Furthermore, the landraces which showed high mineral contents could be used as materials for a biofortification breeding program.

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P60

Maize genomics, genetic engineering and gene editing research and services at the Wisconsin Crop Innovation Center

(submitted by Heidi Kaeppler <hfkaeppl@wisc.edu>)

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Genetic engineering and gene editing systems are critical components in the advancement of maize functional genomics and epigenomics research, and genetic crop improvement efforts. Limitations in current public maize bioengineering systems, including genotype dependency, low efficiency, intellectual property restrictions, and lack of automation and capacity have created a significant bottleneck in the advancement of maize translational genomics investigations. The Wisconsin Crop Innovation Center (WCIC) was established in January , 2017, at the University of Wisconsin to advance basic and applied translational and functional genomic research in crop plants, including maize, through technology development, collaboration, and fee-for-service transformation, gene-editing, and phenotyping activities. Current maize-related research and service activities underway at WCIC include genetic investigation of genotype-dependent tissue culture response, development of novel, transformable maize germplasm, investigation and implementation of high efficiency, genotype-independent transformation and gene editing protocols including meristem and nanoparticle-based systems, and automation of protocols for high throughput production. B73-derived near isogenic lines containing a small genomic segment from A188 imparting embryogenic, regenerable culture response have been developed and recently transformed via Agrobacterium-mediated DNA delivery. Transformation/editing protocols are also being developed for B73 and sequenced maize ex-PVP lines including PH207, PHJ89, PHJ40, PHB47, and LH145. Successful delivery of transgene DNA to specific target cells in maize meristem explants was documented and optimization of the meristem-based selection protocol is underway. WCIC seeks collaborations with partners that have need for large-scale projects. Initial fee-for-service rates for specific services and genotypes are available on the WCIC website with additional services added over time. WCIC is open to discussion of start-up collaboration rates for significant projects with partners that can provide constructs and manage products in the short-term, and have the potential to utilize the capacity of WCIC in the future.

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

P61

Maize *RNA Binding Motif Protein48* (*RBM48*) is required for minor intron splicing and promotes endosperm cell differentiation

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The last eukaryotic common ancestor had two classes of introns that were spliced by different spliceosome complexes. The vast majority of introns, termed U2-type introns, are spliced by the major spliceosome. There are also rare U12-type introns, which are spliced by the minor spliceosome. The biological significance of these rare minor intron genes (MIGs) are not well understood. Mutations in minor spliceosome genes disrupt normal growth and development in both plants and animals. There are relatively few splicing factors that have been shown to be specific to the minor spliceosome. We found that the maize RNA Binding Motif Protein48 (RBM48) is a minor spliceosome factor that functions to promote cell differentiation and repress cell proliferation. Transposon-induced mutations in *rbm48* cause a *rough endosperm* (*rgh*) defective kernel phenotype that alters endosperm cell differentiation to promote aleurone differentiation over basal endosperm transfer cells and embryo surrounding region. Moreover, *rbm48* endosperm is more proliferative in a callus culture system than normal sibling endosperm tissues. RNA-seq and RT-PCR data show that *rbm48* mutants have splicing defects in approximately 60% of MIGs, while U2-type introns are largely unaffected. These developmental and molecular phenotypes are similar to the maize *rgh3* mutant, which also encodes a U12 splicing factor. RBM48 is highly conserved among organisms that retain the minor spliceosome. Protein-protein interactions and co-localization between RBM48, RGH3, and U2 Auxiliary Factor (U2AF) subunits suggests major and minor spliceosome factors may form complexes as part of recognizing introns. Maize RBM48 also shows a conserved interaction with the maize homolog of human Armadillo Repeat Containing Protein7 (ARMC7). Our data predict that RBM48 will have a conserved function in U12 splicing throughout eukaryotes and that a major function of U12 splicing in maize is to promote endosperm cell differentiation.

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P62

Maximizing photosynthesis efficiency in maize

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The theoretical solar use efficiency of photosynthesis is far from being achieved in intensively managed maize cultures, indicating that a large and currently untapped potential for yield increase exists. We aim to improve the efficiency of C_4 photosynthesis by making use of natural variation to elucidate the factors contributing to carbon isotope discrimination ($\Delta^{13}C$) in maize. In C_3 plants, $\Delta^{13}C$ is used as a desirable physiological trait in breeding due to the fact that it is influenced mainly by stomatal conductance, and therefore is an accurate predictor of yield under drought. In C_4 plants, carbon fixation is more complex and the factors contributing to $\Delta^{13}C$ are not well characterized.
A maize introgression library was used to identify genomic regions which influence $\Delta^{13}C$. From this library, near isogenic lines carrying defined segments of the donor parent inserted into the genetic background of the recurrent parent were developed and were analyzed in controlled growing conditions as well as in field trials. The existence of a link between $\Delta^{13}C$, stomatal conductance, assimilation and yield parameters is investigated.
In parallel, a fine-mapping approach is being implemented to narrow down the genomic region responsible for one $\Delta^{13}C$ QTL and allow for selection and characterization of candidate genes.

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P63

Meiotic recombination landscape in maize: impact of chromosome axis and heat stress

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Meiotic recombination plays a prominent role in creating genomic diversity by driving exchanges of genetic information between chromosomes. Studies in maize have revealed that several factors including genomic and epigenomic features shape the landscape of meiotic recombination. However, less is known about how chromosome structure; e.g. the chromosome axis and the synaptonemal complex (SC) regulate the progression and outcomes of meiotic recombination. It has been shown that high-temperature stress affects meiotic recombination patterns and we hypothesize that this interaction is mediated by dynamic changes in chromosome axis. Modulation of temperature may serve as a strategy to manipulate the recombination landscape to allow crossover formation in chromosome regions that exhibit recombination suppression in normal temperature conditions. We are conducting experiments to examine the meiotic recombination landscape and dynamics of recombination events in heat-stressed maize plants, and especially focus on the examination of axis behavior in response to temperature increase. We expect to observe an alteration of meiotic recombination frequency and shift of crossover distribution on the chromosomes, which might be induced by altered axis activity. Our study should contribute to elucidating the role of chromosome axis in meiosis and explaining how environmental factors affect plant genomic diversity by influencing meiotic recombination.

Funding acknowledgement: National Science Foundation (NSF)

P64

Miniature seed 2109 encodes a nitrate transporter protein required for maize seed development.

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The plant nitrate transporter 1/peptide transporter (NRT1/PTR) family comprises low-affinity nitrate transporters and di/tripeptide. Some members also recognize other substrates such as carboxylates, phytohormones (auxin and abscisic acid), or defence compounds (glucosinolates). Little is known about members of this gene family in maize (Zea mays). In this study, we isolated a mutant with reduced seed size from an EMS-mutagenized mutation pool of B73. Gene cloning and characterization indicated that MN2109 encoded a putative transporter that belong to the NRT1/PTR family. This conclusion was based on findings that MN2109 contained a conserved PTR2 region and 12 transmembrane domains, and that the MN2109-GFP fusion protein was localized in the plasma membrane. A detailed function analysis of MN2109 showed that MN2109 was a pH dependent low-affinity nitrate transport protein, MN2109 also acted as a K^+ efflux transporter. The MN2109 gene was specifically expressed in the BETL cell type of maize endosperm. The BETL in mn2109 was impaired compared with its wild type, consistented with this seed phenotype. We found that ZmMRP1 regulated the expression of MN2109 gene. The results of RNA-seq indicated that the metabolic pathway of amino acid biosynthesis、hormone biosynthesis and starch biosynthesis impaired strongly in mn2109 seeds. Our study indicated that MN2109 acted as a nitrate transporter、K^+ efflux transporter, was thus critical for normal BETL cell type and endosperm development in maize.

Funding acknowledgement: Natural Science Foundation of China

P65

Modulation of cold sensitivity and early seedling performance by priming in 27 maize NAM parental inbred lines

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Seedling emergence is an important factor for yield, particularly under challenging planting conditions. In the US corn belt, maize is planted in early spring, as soon as soil temperatures are permissive to germination. At that time, temperatures often drop below normal, which can delay or even kill the seedling. Seed pre-treatments have been shown to improve germination in cold conditions in crops such as rice and cabbage, but are largely unpublished in maize. To assess the effects of pre-treatments on early maize cold tolerance, twenty-seven inbred parents of maize Nested Association Mapping (NAM) population were primed using a synthetic solid matrix and then tested for cold tolerance using a soil-based emergence assay. Primed kernels were incubated at 10°C for 5 days, and then transferred to 24°C for emergence. DSLR cameras were used to capture images every 30 min to obtain emergence profiles of each seedling. Emergence time was determined from the time-lapsed images and multiple measures including final emergence percentage, time to 50% emergence, and emergence rate were extracted for each genotype. The cold treatment reduced total emergence of several genotypes. However, priming pre-treatment protected the sensitive genotypes allowing nearly full emergence. We also used single-kernel near infrared reflectance spectroscopy to determine seed density, weight, oil, protein, and starch for the kernels prior to planting. By combining kernel characteristics and emergence time, we found small, but highly significant correlations between the kernel and early seedling performance. The current status of this project will be presented including the further research results and analysis.

Funding acknowledgement: National Science Foundation (NSF)

P66

Opaque11 is a central hub of the regulatory network for maize endosperm development and nutrient metabolism

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Maize (*Zea mays*) endosperm is a primary tissue for nutrient storage and is highly differentiated during development. However, the regulatory networks of endosperm development and nutrient metabolism in maize remain largely unknown. Maize *opaque11* (*o11*) is a classic seed mutant with a small and opaque endosperm showing decreased starch and protein accumulation. We cloned O11 and found that it encodes an endosperm-specific bHLH transcription factor (TF). Loss-of-function of O11 significantly affected the transcription of carbohydrate/amino acid metabolism and stress-response genes. Genome-wide binding site analysis revealed 9,885 O11-binding sites distributed over 6,033 genes. Using chromatin immunoprecipitation sequencing (ChIP-seq) coupled with RNA sequencing (RNA-seq) assays, we identified 259 O11-modulated target genes. O11 was found to directly regulate key TFs in endosperm development (NKD2 and ZmDof3) and nutrient metabolism (O2 and PBF). Moreover, O11 directly regulates cyPPDKs and multiple carbohydrate metabolic enzymes. O11 is an activator of *ZmYODA*, suggesting its regulatory function through the MAPK pathway in endosperm development. Many stress-response genes are also direct targets of O11. Moreover, eleven O11-interacting proteins were identified, including ZmICE1, which co-regulates stress-response targets and *ZmYODA* with O11. Therefore, this study reveals an endosperm regulatory network centered around O11, which coordinates endosperm development, metabolism and stress responses.

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P67

Phosphoenolpyruvate carboxykinase mutants reveal interaction of C_4 photosynthesis and nitrogen utilization in maize

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Crop breeding efforts will need to increase yields while decreasing the use of non-renewable resources and minimizing detrimental impacts on the soil, water, and air in order to continue to provide food for an increasing world population. Modifications to photosynthesis present an opportunity to increase crop yields. Maize and other C_4 plants evolved a more efficient type of photosynthesis where initial carbon fixation and Calvin cycle activity are spatially separated into the mesophyll and bundle sheath, in order to reduce losses associated with photorespiration. Three subtypes of C_4 photosynthesis exist, and maize utilizes the NADP-malic enzyme (NADP-ME) pathway in combination with the phosophoenolpyruvate carboxykinase (PEPCK) pathway. In the PEPCK pathway, aspartate is used as a transfer molecule between mesophyll and bundle sheath cells, and also acts in nitrogen metabolism, linking the two pathways. Perturbation of the PEPCK pathway is a potential route to increase photosynthetic output and metabolic flexibility for the plant under stress. This project uses a mutant approach to characterize the effect of the pathway at the physiological, biochemical, and transcriptomic levels. *Ds* insertion mutations were identified in the maize PEPCK1 gene, and the pepck1-*Ds* plants were grown under low and high nitrogen in a nitrogen responsive field site during the summers of 2016 and 2017 to determine the effect of the PEPCK pathway under nitrogen stress. At the V8 growth stage, tissue was sampled along the developmental gradient of the leaf, and RNAseq was performed to determine the extent of compensation from the NADP-ME pathway in the mutant. Among agronomic traits, pepck1-*Ds* mutants flowered later, were taller, and had smaller kernels and heavier cobs than controls. The vegetative tissues contained more nitrogen and retained more biomass, indicating that pepck1-*Ds* plants were deficient in nitrogen and sugar remobilization.

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P68

Regulation of cuticle deposition during juvenile vegetative phase in maize

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The cuticle, a hydrophobic film covering the aerial organs of plants, constitutes the first barrier against biotic and abiotic stresses, especially water loss. In addition to its roles in plant protection, cuticle has been shown to have important functions in plant and floral development. The MYB transcription factor *ZmMYB94/fused leaves1* (*fdl1*) is a key regulator of cuticle deposition in maize embryo and seedling. Its action is required to establish a precise boundary between seedling organs, thus preventing fusion between coleoptile, first and second leaf.
To gain insight into the role of FDL1 in seedling development and to obtain information on FDL1-dependent genetic regulation of cuticle biosynthesis, we analysed the biochemical profile of *fdl1-1* mutant and wild type plants at different stages of seedling development. We also performed an Illumina based RNA-sequencing experiment in which transcriptomes from the *fdl1-1* mutant and wild type plants were compared.
Biochemical analysis showed that wax and cutin components were significantly affected in the *fdl1-1* mutant compared to wild type. Moreover, a gene ontology and pathway analysis of the about 1600 differentially expressed genes led to the identification of novel gene candidates implicated in lipid metabolism in maize. Variation in their expression nicely correlated with cuticular differences observed at the biochemical level. Among them, we detected genes involved in fatty acid elongation pathway as well as in wax and cutin biosynthesis and transport. Their expression may be directly or indirectly affected by ZmMYB94. These results along with the analysis of the expression of these genes in response to drought stress conditions will be presented.

Gene / Gene Models described: *fused leaves1 (fdl1)*; GRMZM2G056407

P69

Rhizosphere signaling in arbuscular mycorrhizal symbiosis of maize.

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The arbuscular mycorrhizal (AM) symbiosis is a fascinating mutualistic interaction between roots of most land plants and fungi of the Glomeromycotina. The development of this life-long association starts with reciprocal recognition in the rhizosphere. The interaction proceeds towards extensive root colonization which culminates in the formation of highly branched hyphal structures, the arbuscules, within root cortex cells. It is here where bi-directional nutrient exchange occurs, the basis for mutualism in AM symbioses.
Pre-symbiotic plant-fungal recognition is manifested in a well-orchestrated exchange of signals that leads to reprogramming of both symbionts for the anticipated association. The nature of some of the signals has been discovered in recent years, providing a first insight into the type of chemical language spoken between the two symbiotic partners. Importantly, these discoveries suggest that the dialogue is complex and that additional factors remain to be unveiled. I will introduce some of our recent observations which have led us to propose fundamentally new signaling mechanisms operating during pre-symbiotic communication of this intimate plant-fungal partnership.

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P70

Site specific recombinases: Tools for genetic engineering in maize

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

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P71

Strigolactone biosynthesis in Zea mays (maize)

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Strigolactones are plant hormones regulating a.o. shoot branching, root architecture and secondary stem growth, which are also exuded from the roots into the rhizosphere where they serve as host detection signals for beneficial arbuscular mycorrhizal (AM) fungi. Parasitic plants of the Orobanchaceae family that infect the roots of their host plants have evolved a mechanism to detect strigolactones, and use them as germination stimulants, ensuring that after germination a host is nearby. Parasitic plants are a major threat to agricultural crops and are difficult to control. Multiple structural strigolactone variants have been identified and interesting differences in biological activity such as in mycorrhizal branching and parasitic plant germination stimulatory activities have been observed. In maize root exudate, two canonical strigolactones - 5-deoxystrigol and sorgomol - have been reported. Recently, we demonstrated the occurrence of seven new strigolactones in maize root exudate, of which two were identified as zealactone and zeapyranolactone. The core pathway of strigolactone biosynthesis, up to carlactone, has been identified. However, the enzymes responsible for the formation of specific strigolactones after carlactone, as it now seems three different pathway-branches, are largely unknown.
In this project, candidate genes involved in the strigolactone biosynthetic pathway were identified using co-expression analysis using the known core strigolactone biosynthetic pathway genes (*D27*, *CCD7* and *CCD8*) as bait. In our further analysis, a combination of heterologous expression (transiently in *Nicotiana benthamiana* and other systems), advanced analytical chemistry and transformation will be utilized to elucidate the downstream biosynthetic pathways of strigolactones in maize. This will enable us to create maize lines with altered strigolactone profiles, allowing us to study the biological relevance of the structural diversity in these (rhizosphere) signaling molecules.

Funding acknowledgement: China Scholarship Council (CSC) for funding Li, Changsheng

P72

The maize Oxalyl-CoA Decarboxylase1 acting downstream of Opaque7 is required for oxalate degradation and central metabolism and affects seed nutritional quality

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Oxalate is a widely synthesized organic acid in animals, plants and other organisms. Excessive accumulation of this acid is toxic to cell growth and organ development. Degradation pathways for oxalate in *Oxalobacter formigenes* have been well understood; however, the genes and biochemical reactions for oxalate metabolism in plants have not been fully characterized. Here, we reported the characterization of a new maize opaque endosperm mutant, which exhibits a smaller seed size and shorter plant height compared with the wild type (WT). We cloned this mutant gene and determined that it encodes an oxalyl-CoA decarboxylase1 (OCD1; EC4.1.1.8). *Ocd1* is generally expressed in all detected tissues and was specifically induced by oxalate treatment. The accumulation of oxalate in the ocd1 seed and seedling is significantly higher compared with WT, indicating that the oxalate breakdown is blocked in *ocd1*. The permeability of the seed coat in *ocd1* is also apparently affected. The ocd1 phenotype is similar to that of *opaque7* (*o7*), a classic maize high-lysine mutant. The O7 homologue in Arabidopsis encodes oxalyl-CoA synthetase (EC 6.2.1.8) and is able to catalyze oxalate into oxalyl-CoA and CO2. We purified the recombinant OCD1 protein and determined that it could further degrade oxalyl-CoA, the product of O7, into formal-CoA and CO2. Mutations in *ocd1* caused dramatic metabolic alterations in endosperm. The synthesis of the major storage-protein zeins is significantly reduced and that of non-zeins is compensatorily elevated, leading to a higher lysine level in *ocd1* compared with WT. Targeted metabolomics analyses showed that oxaloacetate, a precursor of oxalate was increased, while NADH (NAD+) which is produced during citric acid cycle was reduced in the *ocd1* endosperm. Our findings demonstrate that OCD1 acts downstream of O7 in oxalate degradation and affects endosperm development, central metabolism and nutritional quality in maize seed.

Gene / Gene Models described: *Oxalyl-CoA decarboxylase*; GRMZM2G175171
Funding acknowledgement: Natural Sciences Foundation of China

P73

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

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Gene regulatory networks are central to all cellular processes. In plants, they help link molecular targets with agronomic traits of functional value including biofuel/biomass production, biomaterials, nutritional health, and stress tolerance. Maize provides an attractive system for investigating the architecture of gene regulatory networks (GRNs) and the underlying gene regulatory grids (GRGs) in cereal crops. To advance the study of regulomics in cereals we developed several years ago the Maize Transcription Factor ORFeome (TFome). The first public release of the maize TFome contained 2,034 clones corresponding to 2,017 unique Transcription factor (TF) and CoRegulator (CR) gene models in recombination-ready vectors (Burdo et al., The Plant Journal. 2014 80:356-66). The entire collection is available through the Arabidopsis Biological Resource Center (ABRC), and has been requested multiple times with a total of over 15,000 clones so far distributed. The potential for this resource to greatly accelerate the discovery of GRNs in plants is demonstrated by its employment to build a protein-DNA-interaction (PDI) network for the phenylpropanoid pathway (Yang et al., 2017. Mol Plant, 10:498–515). As a parallel resource, we developed GRASSIUS (grassius.org) as a gene regulatory information knowledgebase for the grasses. GRASSIUS consists of three interlinked databases that contains a collection of TFs classified into different families (GrassTFDB); transcriptional co-regulators (GrassCoRegDB); and promoter sequences (GrassPROMDB) for maize and other grasses including rice, sorghum, sugarcane, and Brachypodium. GRASSIUS is home to the maize TFome and is being updated to host experimentally determined TF/coregulator protein-DNA interactions (PDIs) and newly annotated maize transcription start sites (TSSs) derived from Cap Analysis of Gene Expression (CAGE) experiments. The utility of GRASSIUS combined with the maize TFome to the scientific community is to accelerate elucidation of regulatory mechanisms that are vital for engineering cereal crops with improved agronomic traits. This project was funded by NSF grant IOS-1125620.

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P74

Tissues lignification, cell wall p-coumaroylation and degradability of maize stems depend on water status

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In the context of both climate change and replacement of fossil resources, water supply and biomass valorization are two urgent issues for providing sustainable systems of production. Maximizing maize biomass valorization is of interest to make biofuel conversion competitive and to increase forage energetic value for animal fodder. One way to estimate these valorizations is to quantify the cell wall degradability. Degradability is influenced by the biochemical composition and structure of the cell wall and also by the lignin distribution in different plant tissues. Recently, few evidences underline that cell wall components and their distribution is also influenced by environmental factors. The aim of this study is to evaluate the impact of water supply on cell wall degradability and composition, and on distribution of lignin in cell type in maize internodes. Dedicated high throughput tools for the quantification of cell wall composition (NIRS predictive equations) and for the distribution of lignin in the different tissues (plugins for image analysis) have been developed. This allowed us to efficiently and accurately phenotype over 528 maize internodes from 11 inbred lines cultivated during 3 years under two contrasted irrigation scenarios in South of France. Overall, our results clearly demonstrate that water deficit induces an upheaval of lignin content and distribution along with a reduced lignin p-coumaroylation which impacts cell wall degradability. Moreover, responses to water supply varied between lines, underscoring biochemical and histological targets for plant breeding.

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P75

Validation and functional characterization of the maize *lateralrootless 1* (*lrt1*) gene

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The monogenic recessive mutant *lrt1* was identified in a segregating F2-generation of an EMS mutagenized B73 population (Hochholdinger and Feix, 1998). The *lrt1* mutant is deficient in lateral root formation in the embryonic primary and seminal roots during early postembryonic development (Husakova et al., 2013).
The *lrt1* gene was fine mapped by a combination of molecular markers and bulk segregant analysis-sequencing. Co-segregation analyses of homozygous wild type and mutant seedlings showed that a candidate gene co-segregated with the mutant phenotype. Confirmation of the candidate gene by independent mutant alleles and CRISPR/Cas9 knock out of the gene is under way.
qRT-PCR experiments of the candidate gene demonstrated that the putative *lrt1* mutation leads to down regulation of *lrt1* expression in primary roots to less than 20% compared to the expression level in wild type primary roots. Furthermore, *lrt1* showed the highest transcript level in the meristematic zone of the primary root, whereas no significant differences in *lrt1* expression were observed between the different root types at different developmental stages.
After confirmation of the candidate gene a more detailed expression analysis by in situ hybridization experiments will be performed. To determine the subcellular localization of LRT1, C- and N-terminal GFP-fusions will be generated.

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Husakova E., Hochholdinger F., Soukup A. (2013): Lateral root development in the maize (Zea mays) lateralrootless 1 mutant. Ann. Bot., 112:417-428.

P76

Why does maize produce benzoxazinoids and transgenic Arabidopsis not?

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Secondary metabolites constitute the chemical defense arsenal of plants. Specific active compounds out of a reservoir of hundreds of thousands are characteristically found in specific plant families. Benzoxazinoids are plant defense compounds mainly produced by grasses. The main benzoxazinoids (BXs) DIBOA and DIMBOA, are synthetized in the seedling and confer resistance against pathogens and herbivores. BX biosynthesis is connected to the tryptophan pathway, all required genes are known from maize and hence theoretically BX biosynthesis can be expressed transgenically in every plant. To assess the effects of BXs on plant physiology, development and defense we are working on a transgenic expression of the whole biosynthesis pathway in *Arabidopsis thaliana*, a dicot with no endogenic BX production.
All maize enzymes of DIBOA biosynthesis, the indole synthase BX1, four cytochromes P450s (BX2 to BX5) and a cytosolic UGT (BX8), could be functionally expressed in *S. cerevisiae* and *A. thaliana*. The expression of the whole pathway chain however so far could not produce the final biosynthesis product DIBOA. In HPLC and LC-MS analyses intermediates of the BX pathway have shown to be readily modified, e.g. by glycosylation in *A. thaliana* and *N. benthamiana*. Modification is by oxygenases and gycosyltransferases. To avoid such modifications of xenobiotics in planta, intermediates have to be captured. This might take place in a protein complex called metabolon. Such a metabolon hypothetically exists in maize. The P450-oxidoreductase is a known nucleation site and the soluble UGT BX8 could also contribute to the complex formation. The impact of these proteins for BX biosynthesis is currently investigated in *A. thaliana* and *S. cerevisiae*. For the evolution of secondary metabolic pathways (ER-)membrane domains that recruit enzyme complexes might be essential: the diffusion of toxic intermediates will be reduced and at the same time the efficiency of the catalysis will be increased.

Gene / Gene Models described: *Bx1, Bx2, Bx3, Bx4, Bx5, Bx8*; GRMZM2G086489
Funding acknowledgement: SFB924, DFG (Deutsche Forschungsgemeinschaft)

P77

Zma-miR169q/NF-YA14 module is involved in salt stress tolerance in maize

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Soil salinity is a major threat to maize productivity worldwide. Plants must adjust their developmental and physiological processes to cope with salt stress. Gene regulation involved by miRNA is essential in these adaptive processes. However, the functions of the miRNA in plant saline stress responses are poorly understood. Here, we identified and functionally characterized a maize salt-tolerance miRNA, miR169q, which targets a nuclear factor Y, subunit A (designated as ZmNF-YA14). The zma-miR169q overexpression line was hypersensitive to salt stress and accumulated more reactive oxygen species (ROS) than the wild-type (WT) under salt stress. Conversely, The zmNF-YA14 overexpression line was resistant to salt stress and accumulated less reactive oxygen species (ROS) than WT. We showed that miR169q was preferentially expressed in root and reduced under salt stress, meanwhile expression of ZmNF-YA14 was increased responding to salt stress. We also showed Superoxide Dismutase (SOD) activity was enhanced in zmNF-YA14 overexpression line compared to WT. RNA-seq analysis revealed that miR169q/NF-YA14 module regulated expression of antioxidant system related genes, which potentially scavenge ROS. Chromatin immunoprecipitation assays revealed that zmNF-YA14 bound directly to the cis-element CCAAT in the promoter of the maize peroxidase 1 (PER1). Our findings highlight the critical role of miR169q/NF-YA14 module as a regulator of salt tolerance in maize.

Gene / Gene Models described: *ZmNF-YA14*; GRMZM2G038303
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P78

ZmbZIP22 is a new transcription factor for 27-kD γ-zein gene

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Zeins are the most abundant storage proteins in maize kernel, affecting maize nutrient quality and kernel hardness. The 27-kD γ-zein is a highly expressed zein that plays crucial role for zein protein body formation. So far, several transcription factors (TFs) (O2, PBF1 and OHPs) have been identified for the 27-kD γ-zein gene transcription. However the complexity of 27-kD γ-zein transcription regulation is not fully revealed. In this study, a new factor binds to the 27-kD γ-zein gene promoter was identified through probe affinity purification and mass spectrometry analysis. This new factor belongs to bZIP type TF (hence named as ZmbZIP22) and is endosperm specifically expressed. ZmbZIP22 can directly bind the ACAGCTCA box in 27-kD γ-zein promoter, and trans-activates the promoter in tobacco cells. The *bZIP22* mutants were generated by CRISPR/Cas9, and the 27-kD γ-zein gene expression was significantly decreased in mutant kernels. ChIP-seq analysis confirmed that ZmbZIP22 binds the 27-kD γ-zein gene promoter in vivo, and also identified additional direct targets of ZmbZIP22. Moreover, ZmbZIP22 can interact with other TFs of 27-kD γ-zein gene, such as PBF1, OHP1 and OHP2, but not with O2. Transactivation assay with combinations of these TFs revealed different interaction mode to the transcription activity of 27-kD γ-zein promoter. These results suggested that ZmbZIP22 is a functional TF for 27-kD γ-zein gene, and it coordinates the regulation of 27-kD γ-zein promoter with other known TFs.

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P79

A phenomics-based dynamic model of growth and yield to simulate hundreds of maize hybrids in the diversity of European environments

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Under soil water deficit, plants limit transpiration by decreasing leaf area to save water for the end of the crop cycle. A large genetic diversity is observed in maize for the processes involved in this response. We aimed to predict which combination of trait values related to leaf growth would be beneficial in the diversity of European environments. For this purpose, we have first analysed the genetic and environmental controls of leaf elongation and widening. A series of experiments revealed that leaf elongation is related to plant water status whereas leaf widening is related to the carbon available to plant. A GWAS analysis also revealed that elongation and widening depend on different alleles. This analysis resulted in a model that allows simulating leaf area in a large variety of environmental scenarios. This model resulted in estimated leaf area and yield that were close to those observed in 15 fields over Europe. The model was then used to determine ideotypes of leaf growth adapted to the different environmental scenarios. Results indicate that sensitive hybrids perform better in southern Europe under rainfed conditions while less-sensitive genotypes perform better in northern Europe or in irrigated fields. However, the best combinations of parameters determined in an unconstrained phenotypic space were not available in the observed genetic diversity. Overall, this study provides elements on where and when a combination of trait values can give a comparative advantage on yield, together with the boundary of possibilities within the current genetic diversity.

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P80

A point mutation in maize *hzMS1* gene causes the meiosis defects in heterozygous mutants

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Meiosis is a key process during plant gametes development, and is precisely regulated by a series genes. And carbohydrate metabolism plays a vital role in the plant growth and development process. Whereas, how the carbohydrate metabolism gene participates the meiotic process is limited known. Here, we studied a new type of maize male sterile mutant *hzms1*, which showed heterozygous male sterile, causing by the chromosome arrested at pachytene stage, while the homozygous mutants had no obvious defects. We mapped hzms1 positionally and found that *hzms1* possesses a point mutation in an invertase coding gene which hydrolyzes sucrose into glucose and fructose. To confirm the cloning result, we constructed transgenic vector *pCambia3300-phzMS1:CDS*^{hzms1} to express the mutated copy in wildtype background, two independent transgenic lines showed the exactly same phenotype to the heterozygous mutant; we also knockout *hzMS1* using CRISPR-Cas9 assay, unexpectedly, the fertility and meiosis process of *hzMS1*^{KO} plants had no obvious difference to wildtype. Collectively, the SNP was the cause of sterility of *+/hzms1* plants. The *hzMS1* was mainly expressed in meiotic anthers, with highest abundance in early prophase microsporocytes. Enzyme activity analysis indicated that the wildtype protein had stringent substrate towards sucrose, while the mutant exhibited no detectable activity. Further experimentations are undertaking to reveal the molecular function of *hzMS1* and its role in maize meiosis regulation.

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P81

Analysis of leaf growth under mild drought in maize recombinant inbred lines.

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Plants absorb light energy with their leaves, and convert the energy to carbohydrates, making leaf growth an important yield component. Two main determinants for leaf size are the maximal growth rate (leaf elongation rate; LER) and the duration of the growth period (leaf elongation duration; LED). Here, we studied how changes in environmental conditions affect LER and LED by applying a mild drought stress to a selected panel of a B73xH99 recombinant inbred line (RIL) population. The mild drought stress negatively affects the maximal growth rate, but remarkably prolongs the duration of growth, suggesting that the prolonged growth duration compensates, at least partly, for the reduction in growth rate. To test the hypothesis that the prolonged duration of the reduced growth rate in mild drought treated maize plants represents a pausing that can be restored to 'normal' growth rates when water becomes available, we re-watered drought treated plants at different time points during their growth. Depending on the growth phase of the leaf upon re-watering, growth could be either fully restored by increasing the maximal growth rate or partially restored by prolonging the duration of growth. These phenotypic analyses were complemented with RNAseq transcriptome data to have a molecular view on the recovery of LER and/or prolongation of LED after re-watering. The observed differences in the recovery potential of plants is a useful trait for breeding towards drought tolerance.

Funding acknowledgement: European Research Council (ERC)

P82

Bioremediation analysis of water pollutants and pathogens within household water in rural south India

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Unsafe drinking water is recognized as a leading factor in diarrheal diseases, responsible for about 5 million annual deaths globally, six hundred thousand deaths alone coming from India. An international field study was fashioned to document the techniques used by an the Indian Social Service Institute in Southern India to construct, develop, assemble and distribute bio-sand filters (BSFs), a cost-effective water filtration system, for at home use. The field study used BSFs to combat microbiological contaminants in household water by using a mass of organic and inorganic charged compounds that create an environment used to remediate contaminated water. More importantly, this study assessed the logistics and efficiency of BSFs in Pudukkotai, Tamil Nadu. The three month study created concrete BSFs and filled them with sand and gravel from the surrounding environment to create a naturally occurring biological filtration system. Laboratory analyses were performed in five day increments on water samples using indicator strips that tested for pH, NO3-, NO2-, PO4-3, alkalinity and water hardness. An increase or decrease in nutrient levels over time from this analysis would indicate the growth or decay of a microbiological community within the BSF. Water analysis did not indicate a growth of a biological layer, called Shmutzdecke, which indicates the method used for assembly of a BSF must be configured for greater efficacy. The study is on-going and once an effective system of configuration is produced this BSF project is expected to construct and distribute 50 filters within the year for the villages located within Pudukkottai.

P83

Characterization and cloning of *needle1 (ndl1)*, a temperature sensitive mutant affecting reproductive organogenesis

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ndl1 is a temperature sensitive mutant that develops tassels with fewer branches and spikelets, and ears with unorganized kernels and partially barren tips. When grown at higher temperature, *ndl1* affects root development as well. Scanning Electron Microscopy and *in situ* hybridizations with early markers for organogenesis suggest that suppressed bract and axillary meristem initiation are defective in *ndl1* inflorescences.
By a combination of map-based cloning and BSA RNA-seq approaches, we identified a candidate gene encoding a mitochondria-localized protein with a missense mutation in a highly conserved amino acid. The homologous genes in Arabidopsis have been reported to affect the function of the electron transport chain. In *ndl1* inflorescences we observed high expression of the *ALTERNATIVE OXIDASE-2 (AOX2)* gene, a marker for defects in the electron transport chain, whose expression is not affected in other mutants similar to *ndl1*.
We are currently following several complementary approaches to confirm the candidate gene. We developed maize transgenic lines expressing the candidate gene from its native promoter and complemented the *ndl1* phenotype. At the same time, we are employing *CRISPR/Cas9* based approaches to generate new lesions in the candidate gene, and to investigate the phenotype of loss of function mutants in both maize and Arabidopsis.

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P84

Characterization of a novel Kinesin protein required for Ab10 meiotic drive

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Originally discovered 75 years ago, maize Abnormal Chromosome 10 (Ab10) lines display meiotic drive where the segregation ratios of alleles linked to chromosome knobs are significantly altered from the expected 50:50. It is estimated that meiotic drive in maize has had evolutionary consequences on a genome-wide level by affecting segregation of alleles near knobs. The cytological cause of meiotic drive is neocentromere activity during female meiosis where knobs are directed towards the poles and arrive before centromeres. During female meiosis in plants, only the single lowest of the four cells survives and neocentromere activity favors the knob-containing chromosome to migrate into what will become the functional megaspore. Knobs are composed of two distinct tandem repeat arrays termed knob180 and TR1. Ab10 itself contains genes required for meiotic drive and neocentromere activity, but prior to this work none of these genes had been described. We show that the distal tip of Ab10 contains the eight-member *Kindr* (*Kinesin driver*) gene family and that *Kindr* is necessary for meiotic drive and knob180 neocentromeres to occur. *Kindr* encodes a functional minus end-directed Kinesin-14A homolog protein that is present in meiotic anthers and ears of Ab10 but not N10 plants. Furthermore, immunolocalization studies show that KINDR colocalizes specifically with knob180 knobs during male meiosis. Despite colocalization, KINDR does not undergo a direct protein-DNA interaction with knob180 DNA sequences. In summary, our work has identified a novel Ab10 kinesin that contributes to meiotic drive by facilitating meiotic neocentromere activity of the knob180 knobs.

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P85

Characterization of meiotic defects in the maize *nrf4* mutant

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Apomixis is a form of asexual reproduction through seeds that leads to formation of offspring that is genetically identical to the mother plant. Introduction of apomixis into the crop plants has the potential to revolutionize agriculture by providing an ideal tool to fix complex genotypes. If applied to including F1 hybrids, the heterosis effect could be stably propagated over many generations.

Through a genetic screen in maize, we identified a recessive mutation - *n*on-*r*eduction in *f*emale 4 (*nrf4*) – that mimics aspects of apomixis. Homozygous mutants produce up to 95% of unreduced embryo sacs and in a fraction of those also no recombination occurred, mimicking apomeiosis, the first step of apomixis. To identify the exact meiotic defects in the apomeiotic *nrf4* mutant, we quantitatively analyzed the localization of meiotic markers in *nrf4* female meiocytes in comparison to wild-type meiocytes by whole-mount immunostaining [She et al. (2018) Methods Mol Biol 1675:443-454]. To shorten the generation time to facilitate this study, we introgressed the *nrf4* allele into the fast-flowering mini-maize background [McGaw et al. (2016) Genetics 204:35-42]. Cytological analyses of female meiosis in the *nrf4* mini-maize mutant by immunostaining revealed multiple defects, for instance in the installation of key elements of the synaptonemal complex, the expression of a meiosis-specific gene controlling the cell cycle, misalignment and decondensation of chromosomes on the metaphase plate, as well as the distribution of H3Ser10p during metaphase I. Our findings suggest that chromosome pairing, synapsis, and segregation during meiosis I are affected in the *nrf4* mutant.

P86

Characterization of RAMOSA3 putative nuclear interactors and their role in inflorescence development in maize

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The classical maize mutant *ramosa3* (*ra3*) shows branches in female inflorescences and increased branching in male inflorescences; *RA3* encodes a trehalose phosphate phosphatase enzyme, specifically expressed at the base of inflorescence axillary meristems. RA3 is localized in nuclear and cytoplasmic speckles, and a RA3 catalytic-dead transgene can partially complement *ra3*, suggesting additional roles other than catalysis of trehalose synthesis. Here we ask if specific interactors can explain mechanistically how RA3 inhibits inflorescence branching and if RA3 functions in gene expression regulation.
To explore these hypotheses, we screened for RA3 physical interactors using yeast-two-hybrid screening and identified some nuclear localized proteins. We focused on two candidates involved in gene regulation: *Zea mays* SCAFFOLD ATTACHMENT FACTOR B (*Zm*SAFB) and *Zea mays* VASCULAR PLANT ONE–ZINC FINGER (*Zm*VOZ). SAFB has been described in animal systems as an RNA Recognition motif (RRM) protein involved in transcriptional co-repression and splicing, but its functions in plants are not defined yet. In *Arabidopsis*, VOZ1 and VOZ2 redundantly link flowering time with light sensing, with no obvious role in meristem branching or determinacy.
To characterize their role in the RA3 pathway, we first identified homologs for *Zm*SAFB and *Zm*VOZ in the maize genome, and CRISPR mutant alleles were generated. Genetic interactions with RA3 and our CRISPR alleles showed *Zmsafb* enhances the *ra3* phenotype. Additionally, *Zm*SAFB-mRFP1 transgenic lines showed constitutive expression in a punctate pattern in nuclear compartments, and partial co-localization with RA3 and the active form of RNA POLII, suggesting a role of *Zm*SAFB in transcriptional regulation. *Zm*VOZ1-RFP and YFP-*Zm*VOZ5 lines have also been generated, and their localization patterns are under analysis. These tools will allow us to confirm physical interactions with RA3 *in planta*, and by using cell biology, biochemical, genetic and genomic approaches we will determine how *Zm*SAFB and *Zm*VOZ function in the RA3 pathway.

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P87

Characterization of the maize *lil1-1* mutant defective in the Brassinosteroid C-6 oxidase

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The Brassinosteroids (BRs) hormones play an essential role in plant growth and development and mediate the plant response to different environmental stimuli. BR biologically active molecules castasterone (CS) and brassinolide (BL) are synthetized from sterols through a complex pathway, whose characterization has been achieved with biochemical analysis as well as with the isolation and subsequent characterization of mutants impaired in specific steps. The main feature of BR deficient mutant plants is their dwarf growth stature, due to reduced internode elongation. Here we present the characterization of a mutant allele of the *brassinosteroid-deficient dwarf1* (*brd1*, GRMZM2G103773) gene, referred to as *lilliputian1-1* (*lil1-1*), which was isolated through transpositional mutagenesis.
The *brd1* gene, encoding a brassinosteroid C-6 oxidase, controls the final steps of the BR pathway, while the *nana plant1-1* (*na1*) gene, encoding a 5α-reductase enzyme, is involved in earlier steps. Detailed phenotypic analysis of F2 progenies segregating for both *na1-1* and *lil1-1* mutants showed that the *lil1-1* mutation causes a more severe reduction in plant elongation and is epistatic to *na1-1*. This observation suggests that an additional *na1*-independent branch, leading to the production of CS precursors, might be present in the maize BR pathway.
The lil1 mutant phenotype includes several characteristics, such as reduction in plant height, dark green leaves and feminized male flowers, which are common to all BR related mutants. Our study highlights the presence of additional traits not previously reported, including altered root gravitropic response and epicuticular wax deposition, which are caused by BR deficiency in this mutant. In addition, by comparing *lil1-1* mutant and wild type individuals grown in well-watered and in drought stress condition, we assayed the effect of BR deficiency on the response to drought stress.

P88

Characterizing the function of kinectin in plants via Zea mays and Arabidopsis thaliana

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Establishment and maintenance of the division plane is essential for proper growth and development in multicellular organisms. TANGLED1 (TAN1) promotes proper division plane orientation and localizes to the future division site during mitosis. In *Zea mays*, *tan1* mutants have disorganized cell architecture that leads to rough leaf texture and short stature. A yeast-2 hybrid experiment identified KINECTIN1 (KNN1) as a candidate TAN1-interacting-protein. Orthologs of this gene in animals encode integral endoplasmic reticulum (ER) proteins that regulate the movement of motor proteins. We chose to investigate KNN1 for its potential role in the division plane orientation and contributions to plant growth. Our hypothesis is that KNN1 is an ER localized protein that contributes to proper growth and development through TAN1 interaction. Maize *knn1* mutant alleles were generated using *CRISPR*/Cas9. Homozygous loss-of-function *knn1* single mutants have apparently normal ER morphology with no obvious cell division plane orientation defects. KNN1-YFP colocalized with the preprophase band, spindle and phragmoplast during mitosis and possibly co-localized with the ER, during interphase in *Arabidopsis thaliana*. Our preliminary findings suggest that KNN1 may be required for growth in maize.

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P89

CRISPR/Cas9 gene editing in the maize inbred line B104

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CRISPR/Cas9-mediated gene editing is revolutionizing plant breeding. Plant transformation and regeneration are however new bottlenecks in efficient and genotype-independent application of gene editing. Here we report on CRISPR/Cas9-mediated gene editing to create loss-of–function alleles in the maize inbred line B104. Recent reports describe the use of the morphogenic regulators OVULE DEVELOPMENT PROTEIN (ODP2) and WUSCHEL (WUS2) to enhance maize transformation. We generated Golden Gateway-compatible building blocks allowing combining these transcription factor modules easily with CRISPR/Cas9. First results with these constructs in B104, PHP38 and PHN46 will be discussed. Use of these morphogenic regulators will allow shorter procedures and higher throughput for generating loss-of–function alleles, supporting functional genomics in maize.

Gene / Gene Models described: *ODP2, WUS2;* GRMZM2G14638, GRMZM2G028622

P90

Deposition of lipids in starch granules of maize endosperm is closely related to zein synthesis and programmed cell death

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Presence of lipids within starch granules is a specificity of cereal endosperms. Although these lipids account for only 0.7-1.5% of starch dry mass, they have strong impacts on assembly and properties of cereal starches. Besides, cereal endosperms undergo a programmed cell death (PCD) during their development and its impacts on the deposition of starch lipids is unknown. Furthermore, in contrast with chloroplasts, limited information is available on amyloplast lipid homeostasis. Using Raman microspectrometry and MALDI mass spectrometry imaging, we established that the spatio-temporal deposition of storage proteins, i.e. zeins and the major starch lipids, i.e. lysophosphatidylcholines (lysoPC), are closely related. Since zeins are synthesized in the endoplasmic reticulum (ER), this suggests, in regard to what is known about chloroplast lipid homeostasis, that starch lysoPC could derive from the ER. Transcriptomics strengthened this hypothesis since homologous genes of the Arabidopsis ER-chloroplast lipid trafficking are also expressed in maize endosperm. However presence of lysoPC within starch granules means that it is translocated through outer and inner amyloplast envelope membranes in contrast to the mechanisms described in chloroplasts. In addition, during endosperm development, palmitate content of amyloplast galactolipids decreased, in relation with a preferential trapping of palmitoyl-lysoPC by starch carbohydrates, suggesting a role of lysoPC in galactolipid synthesis. Finally, a gene encoding a cytosolic patatin-like phospholipase A2 was overexpressed in the endosperm periphery. This gene, induced by PCD in Arabidopsis, is a relevant candidate for the production of lysoPC from ER membranes. Altogether, our results led us to propose a model where ER-amyloplast lipid trafficking directs the lysoPC in two routes, one towards starch granules and the other towards galactolipid synthesis. Finally, lysoPC gradients fit well with those of endosperm vitreousness and new gene candidates were identified that could be tested to improve this important quality trait of maize crop.

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P91

DiSUMO-LIKE interacts with cell cycle and RNA pathways, and regulates early embryo development in maize

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Embryogenesis in maize and other flowering plants is initiated by an asymmetric zygote division, generating two daughter cells that are the precursors of various cell lineages. Until now, little is known about the molecular players regulating activation and progression of zygote development, establishment of asymmetry and the cell plate formation. We have found that a cereal-specific ubiquitin-like modifier diSUMO-LIKE (DSUL) functions in early embryo development in maize. Introduction of a *DSUL*-RNAi construct by sperm cells inhibits completion of cytokinesis and generates non-separated zygotic daughter nuclei or multinucleate embryonic cells lacking cell plates. DSUL localizes to the cytoplasm, nucleus and accumulates in the cell division zone. Identification of DSUL targets suggests predominant roles of DSULylation in regulating the translation machinery and in cell plate formation. A comparison of DSUL and SUMO1 localization patterns during the cell cycle and of their target proteins reveals functional diversification between these two SUMO-family modifiers in maize.

Gene / Gene Models described: *DiSUMO-LIKE (DSUL)*; GRMZM2G073404
Funding acknowledgement: German Research Foundation (DFG)

P92

Diversity in the surface lipid composition of maize silks among Wisconsin Diversity Panel inbreds in two environments

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Extracellular surface lipids are specialized metabolites synthesized by epidermal cells that accumulate on aerial portions of plants and provide a protective barrier against both biotic and abiotic stresses. Maize silks are rich in surface lipids, accumulating predominantly hydrocarbons and minor amounts of fatty acids, which are the end-point and precursor metabolites of the hydrocarbon biosynthetic pathway, respectively. The underlying genetic networks that organize the synthesis and localization of these surface lipids are not fully understood. To dissect the genetic network, we are profiling the surface lipids on silks from 500 genetically diverse inbred lines of maize that comprise the Wisconsin Diversity (WiDiv) Panel. Silks that had emerged from encasing husk leaves were sampled in both Minnesota and Iowa during Summer 2016 and extracted surface lipids were profiled via gas chromatography and flame ionization detection. A high-throughput metabolomics analysis pipeline has revealed a set of approximately 50 unique lipid metabolites. Across the 50% of the WiDiv panel profiled to date, a 14-fold range in total metabolite accumulation was observed. Herein, we will present a comparison of metabolome compositions from silks of inbreds profiled in these two distinct environmental conditions. Potential diversity in the metabolic network will be probed by assessing fatty acid precursor and hydrocarbon product relationships, providing insight into the mechanisms responsible for their synthesis. Future work will include genome wide association studies to understand the underlying genetic networks controlling these metabolites, and how this genetic network interacts with environmental perturbations.

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P93

early delayed kernel 1 (*edk1*) encodes a microtubule-located protein essential for early endosperm development in maize

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Maize (*Zea mays*) is a highly productive crop that is widely used as food and feed. The seed size and plant height are two critical agronomic traits related to the yield and are under control of multiple complex factors. In Arabidopsis and rice, several signal pathways regulating the seed size have been extensively elucidated, while the related research in maize still lacks, particularly at early seed development stage. Using EMS mutagenesis, we screened a small seed mutant that is developmentally delayed at early stage (*early delayed kernel 1*, *edk1*). The mutant plant is shorter compared to the wild type (WT, A619). We cloned this gene and determined that the mutation in *edk1* resulted in a CGA → TGA transition, leading to a premature stop codon in the coding sequence. Cytohistological examination revealed that the reduced seed size in *edk1* was due to the decreased cell number rather than cell size in the endosperm. Developmental defects in the cell wall ingrowth (CWI) at the basal endosperm transfer layers (BETL) were also observed, which may partially contribute to its small seed size. *edk1* was determined to be located on cortical microtubules. Spatial and temporal expression analyses revealed that *edk1* was most abundantly expressed between 2-3 DAP, preferentially enriched in the syncytium area during the endosperm development, indicating that EDK1 plays an important role in nuclear divisions at syncytial stage. We propose that lack of EDK1 may affect the syncytial (and cellularization) development, which in turn reduces the cell number and as a consequence the seed size in the later endosperm development.

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P94

fun is: pleiotropy, unknown genes and double mutants

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Feminsed upright narrow (*fun1*) is a pleiotropic maize mutant that has a loss of auricle and thus a leaf angle defect, a narrowing of leaves, and feminisation of tassel flowers and architecture. The *fun1-1* mutation contains a premature stop codon in the last exon of a gene of unknown function that is conserved primarily as single copy across angiosperms with high conservation in grasses. The non-complementing *fun1-2* allele also contains a premature stop codon in the same gene. Though neither functional nor predicted annotations of the gene or its homologues exist in the databases, transient expression of YFP clones in *Nicotiana benthamiana* suggests protein localisation to the nucleus. Y2H and RNAseq analysis have identified putative interactors of the protein and sketched the genetic landscape of the mutant. An additive phenotype in the *lg2;fun1* double mutant suggests that *fun1* exists in a separate, later pathway than LG2. The extreme synergistic phenotype of the *WAB;fun1* double and examination of the *WAB;fun1;lg2* triple has allowed a deeper interrogation of the *lg2;fun1* interaction, showing that these genes operate in different areas of the ligular region. On the other hand, the *lg1;fun1* double loses the partial recovery of ligule seen in the *lg1* single mutant, suggesting that FUN1 also plays a role early in the ligule's development, in the same ligular region as LG1. Feminisation in *fun1* is a result of a failure to abort the young silk, and, while the *sk1;fun1* tassel loses silks, tassel architecture and florets continue to show secondary feminine traits implying that feminisation of *fun1* is not related to jasmonic acid deficiencies. *fun1* is epistatic to *Bin1-RNAi* transgenic mutants, implying that the *fun1* mutation may block the brassinosteroid response pathway, while a synergistic phenotype in the *dwarf1;fun1* double mutant suggests that FUN1 doesn't interact with the gibberellic acid pathway.

Gene / Gene Models described: *GRMZM2G323353, GRMZM2G110242, GRMZM2G036297, GRMZM2G060216, GRMZM2G021786*; Zm00001d039435
Funding acknowledgement: National Science Foundation (NSF)

P95

Functional analysis of pollen-specific RALFs during reproduction in maize

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Small secreted peptides can be classified into two major groups, CRPs (cysteine-rich peptides) and non-CRPs. Previous studies have shown that members of various CRP sub-classes are involved in different steps of the double fertilization process of flowering plants. To investigate the roles of CRPs during maize reproduction, we performed RNA-seq analysis to identify CRPs with specific expression pattern during pollen tube growth and fertilization. We identified three genes encoding rapid alkalinization factor (RALF) CRPs, which are highly and exclusively expressed in germinated pollen tubes. To understand the function of these pollen-specific RALFs during reproduction in maize, *RALF*-RNAi lines were generated. During *in vitro* germination tests, pollen tubes from down-regulated lines were less stable and burst much faster compared with wild type pollen tubes. The effect of pollen cell wall instability and its consequence is now investigated *in vivo*. Functional studies of RALFs in *Arabidopsis thaliana* revealed that peptides of this gene family are involved in multiple aspects of plant growth and development. For example, it has been shown that RALF1 interacts with the receptor-like kinase FERONIA in Arabidopsis root development and immune signaling(Bergonci *et al.*,2014;Stegmann *et al.*,2017). Pollen expressed RALF4/19 can interact with FERONIA-like receptor like kinases ANXUR1/2 and BUPs1/2. Interaction of these receptors with ovule expressed RALF34 induces burst of growing pollen tubes(Ge *et al.*,2017). Based on sequence alignment and expression pattern comparisons, several putative FERONIA homologs were found in maize silks in a pollination-specific expression manner. Knocked-out mutant lines with CRISPR-Cas9 of above mentioned pollen specific *RALFs* from maize and possible receptor-like kinases have been generated and are currently being transformed. Moreover, we now study the specificity of interactions between maize and Arabidopsis RALFs and their corresponding receptors.

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P96

Functional analysis of the heterotrimeric Gγ subunits in maize

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Heterotrimeric G proteins are membrane-associated proteins involved in the transduction of extracellular signaling by activating downstream effectors. However, it is a widely accepted fact that the mechanisms of G protein activation and signaling in animals compared to plants are fundamentally different. G protein signaling in plants affects a variety of physiological and developmental processes, which is also manifested in the pleiotropic phenotype of heterotrimeric subunit mutants in various plant species, including Arabidopsis, rice and maize. On average plants share one canonical Gα, one Gβ and multiple Gγ subunits. which can be classified into three types: Type A Gγ subunits resemble the canonical type also present in animal systems, possessing a C-terminal prenylation motif. Type B subunits are structurally similar to Type A, but lack the C-terminal prenylation motif. Type C subunits are characterized through the presence of a long, cysteine rich C-terminal region.

The Gγ subunits have been shown to play important roles in development. Especially the Type C Gγ subunit, DEP1, in rice has been shown to affect cell proliferation and inflorescence development.
As part of a functional genomics approach to elucidate the function of maize Type C Gγ subunits, we are analyzing the tissue specific and subcellular expression pattern of the Type C maize Gγ subunits. We will present *in situ* hybridization data in developing inflorescences. Initial data suggest an altered expression pattern in Gα mutants compared to wild type. Furthermore initial subcellular localization data of YFP-tagged constructs transiently expressed in tobacco leaves will be shown.
Additionally UniformMu insertion lines and Maize Targeted Mutagenesis mutants for the DEP1-like Gγ subunits were isolated to identify the role of the Gγ subunit in plant development.

Funding acknowledgement: German Research Foundation (Deutsche Forschungsgemeinschaft DFG)

P97

Functional characterization of male gametophyte genes under heat stress

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Genome-wide expression analysis is a powerful tool to identify genes affected by environmental stresses during developmental programs. In maize, male gametogenesis is generally considered to be especially sensitive to various stresses. Using the Leaf Collar Method (Begcy and Dresselhaus, 2017) we have dissected transcriptional expression patterns from discrete pollen developmental stages. To understand mechanistically how heat stress affects male gametophyte, we imposed a moderate (35°C) heat stress treatment on developing pollen at various stages including the unicellular and bicellular stage. Heat stress resulted in shrunken pollen and reduced pollen viability. We identified a set of genes including transcriptional regulators with a potential role to mitigate the effect of heat stress during pollen development. To functionally characterize differentially expressed candidate genes, we are currently generating maize CRISPR-Cas9 lines to study their function during male gametophyte development under heat stress. Our findings suggest that several of these genes are preferred targets for heat stress. Engineering such candidate genes could potentially help in the future to improve thermal resilience in monocot plants.

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Begcy K, Dresselhaus T (2017) Tracking maize pollen development by the Leaf Collar Method. Plant Reprod 30: 171-178

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P98

Functions of PP2A phosphatases in Arabidopsis stomatal development

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Stomata guard cells control gas exchange between plants and the atmosphere. In Arabidopsis, stomatal development is regulated by peptide ligands, membrane receptors, a mitogen-activated protein kinase (MAPK) cascade, and a set of transcriptional factors. The initiation of the stomatal lineage requires the activity of the bHLH transcriptional factor SPEECHLESS (SPCH) and its partners. Multiple kinases, including MAPK3/6, the GSK3-like kinase BIN2 and Cyclin-Dependent Kinase A;1, have been identified to regulate SPCH protein stability through phosphorylation, yet no phosphatase has been characterized thus far.
In this work, we identified PP2A phosphatases as novel positive regulators in Arabidopsis stomatal development. PP2As form heterotrimeric complexes, each of which is composed of a scaffolding subunit (A), a regulatory subunit (B), and a catalytic subunit (C). The single and double T-DNA insertional mutations in the three PP2A-A genes exhibited decreased stomatal production, and both RNAi knocking down and CRISPR-Cas9 knocking out all three PP2A-A subunits resulted in similar effects. Consistently, the PP2A-specific inhibitor Cantharidin (CT) treatment of Arabidopsis seedlings suppressed stomatal formation. Furthermore, we show that CT specifically suppressed the SPCH protein abundance. Genetic analyses indicated that PP2As act downstream of the MAPK cascade, but upstream of SPCH. Considering PP2As were reported to participate in the brassinolide (BR) hormone signaling, and BRs were found to regulate stomatal development through the BIN2 kinase, we are now testing the potential connection between BR signaling and PP2A function. Our results suggest that PP2As promote stomatal development through stabilizing the SPCH protein.

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P99

Genetic control of maize floral development

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Flowers have an essential role in plant reproduction and also produce fruits and seeds, which are a major food source. Grass flowers (called florets) have a highly derived morphology and in addition to stamens and carpels contain the grass-specific organs lodicules, palea and lemma. Maize spikelets contain two florets, which are the product of the upper and lower floral meristems. In the tassel, both florets develop to produce two male florets that are morphologically indistinguishable at maturity. In the ear, the lower floret aborts resulting in spikelets with a single floret. To understand the gene regulatory networks that function in floral development, we used lasercapture microdissection coupled with RNA-seq to identify genes specifically expressed in the upper and lower floret. Approximately 600 genes are differentially expressed between the upper and lower floral meristems in ears (FC>2; q<0.05) and are enriched for genes involved in transcriptional regulation, development and hormone metabolism.

We have also begun analysis and positional cloning of the classical semi-dominant mutant, *Polytypic1 (Pt1)*. The *Pt1* phenotype varies significantly depending on inbred background. In the A619 inbred background, *Pt1* heterozygotes have a mild phenotype and are often male and female fertile, whereas in the B73 inbred background, *Pt1* heterozygotes are female sterile and tassels shed little pollen. Floral meristems in *Pt1* mutant ears are indeterminate and make abnormal floral organs. Interestingly, *Pt1* homozygotes often have pin-like inflorescences that produce few or no lateral primordia. *Pt1* maps to bin 6.05 and we plan to identify candidate genes using RNA-seq. Together, these approaches will uncover new genes and regulatory networks that function in grass floral development.

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P100

Genetic control of stomatal conductance in maize and conditional effects to water deficit and evaporative demand as revealed by phenomics

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Plants tend to decrease transpiration under water deficit and/or high evaporative demand by closing stomata. Stomatal conductance is central for the trades-off between hydraulics and photosynthesis. We aimed at deciphering its genetic control and that of its responses to evaporative demand and water deficit, a nearly impossible task with gas exchanges measurements. Whole-plant stomatal conductance was estimated via inversion of the Penman–Monteith equation from data of transpiration and plant architecture collected in a phenotyping platform. We have analyzed jointly 4 experiments with contrasting environmental conditions imposed to a panel of 254 maize hybrids. Estimated whole-plant stomatal conductance closely correlated with gas-exchange measurements and biomass accumulation rate. Sixteen robust quantitative trait loci (QTLs) were identified by genome wide association studies and co-located with QTLs of transpiration and biomass. They accounted for 58% of the additive genetic variance and 40% of the genotype × environment interaction. Light, vapour pressure deficit (VPD), or soil water potential largely accounted for the differences in allelic effects between experiments, thereby providing strong hypotheses for mechanisms of stomatal control and explaining part of the observed genotype × environment interaction. Light positively affected the allelic effects of three QTLs (e.g. $R^2 = 0.74$), whereas VPD and water deficit negatively affected the allelic effects of other four QTLs. The combination of SNP effects, as affected by environmental conditions, accounted for the variability of stomatal conductance across a range of hybrids and environmental conditions ($R^2 = 0.86$). This approach may therefore contribute prediction of stomatal control in diverse environments and to breeding for water efficient maize.

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P101

Genetic mechanisms for bud suppression during maize domestication

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Many domesticated crop plants have been bred for reduced axillary branching compared to their wild ancestors. In maize this has been achieved through selection for gain of function alleles of the TCP transcription factor teosinte branched1 (tb1) nearly ten thousand years ago. Despite its importance, the precise genetic mechanism for how tb1 was able to achieve bud suppression is unknown. By raising an antibody to TB1 and performing chromatin immunoprecipitation and high through-put sequencing (CHiP seq) on very young axillary bud tissue, we identified the genetic pathways necessary for TB1 function. For example, TB1 targets several hormone pathways to effect axillary bud suppression, including auxins and gibberellins, but also targets genes controlling sugar metabolism whose products are necessary to feed the growing bud. Interestingly, TB1 also targets several previously described genes in the domestication pathway including teosinte glume archeticture1 (tga1) and grassy tillers1 (gt1). This fact places tb1 near the top of the domestication hierarchy, demonstrating the critical importance of this gene during the domestication of maize from teosinte.

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P102

Genome-wide analysis of small RNA-controlled gene networks in leaf development

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In plants, stem cell niches serve as a stable source of cells for postembryonic growth and development. The shoot apical meristem (SAM) gives rise to all aerial organs of a plant, and its activity throughout the plant's lifetime therefore has to be tightly controlled in a spatiotemporal manner. To gain insight into gene regulatory networks behind stem cell maintenance and organogenesis, we generated a high-resolution gene expression atlas of 12 distinct domains within the vegetative maize shoot apex using laser microdissection and RNA deep sequencing. We also generated small RNA sequencing data that informs on the role of miRNAs in the maize shoot apex. Together these data reveal a subfunctionalization of miRNA family members across the SAM subdomains, and the regulation of miRNA accumulation in the stem cell containing SAM tip and vasculature. In addition, miRNA degredome sequencing data, combined with information from the SAM atlas, predicts the presence of mechanisms that further fine-tune the accumulation and activity of select small RNAs to regulate key meristem genes.

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P103

Grain abortion under drought in maize: expansive growth and hydraulics also matter

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Yield maintenance under drought in maize (*Zea mays*) requires the rapid extension of styles and stigma (silks) that collect pollen. We have shown that the control of grain set under moderate water deficits similar to those in the field result from a developmental process linked to the timing of silk growth, in opposition to the common view that abortion is linked to the sugar metabolism in ovaries. A switch to abortion occurs 2-3d after first silk emergence in water-stressed plants, when silk growth stops simultaneously for all ovary cohorts, and explains abortion rates in different treatments and positions on the ear. Analyses of transcripts and metabolites indicate that the first molecular events occur in silks rather than in ovaries, and involve genes affecting expansive growth rather than sugar metabolism. Sugar availability is preserved in ovaries until the switch to abortion, and the disruption of carbon metabolism only occurs afterwards. Hence, changes in metabolite contents, transcript amounts and enzyme activities involved in ovary sugar metabolism would be a consequence rather than a cause of the beginning of ovary abortion. Patterns of silk growth responses to environment share common features with those of leaf growth with both kinetic and genetic evidences. These findings have large consequences for breeding drought tolerant maize and for modelling grain yields under drought.

Oury et al (2016) *Plant Physiology* 171: 986-996 and 171: 997-1008
Turc et al (2016) *New Phytologist* 212: 377–388
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P104

Heat stress induced male sterility during pollen development in maize

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Shifts in the duration and intensity of heat stress are expected to have a detrimental effect on plant development, reproduction and yield. Increased male sterility has been reported and several factors associated to variations in optimal temperatures have been shown to negatively affect male gametophyte (pollen) development. How the specific stages of pollen developmental respond to increased temperatures is not understood. To elucidate the mechanistic basis of heat stress sensitivity causing reduced male gametophyte viability and partially arrested development, we exposed maize plants to a moderate (35/30 °C day/night) heat stress for 48 hours at various stages of pollen development. Physiological and biochemical analysis of maize plants heat stressed at the tetrad stage of pollen development showed less pollen grains adhered to anthers, reduced pollen viability and germination capability. Pollen grains also contained less starch. Next, we analyzed changes in gene expression pattern. Approximately 300 genes were differentially expressed under heat stress condition at the tetrad stage. Gene Ontology analysis of differentially expressed genes revealed that around 40 % of the genes found are involved in metabolic processes. Detailed analysis showed that biosynthesis pathways used to generate energy and lipids are especially affected by increased temperatures during the tetrad stage leading to male sterility and thus affecting yield.

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P105

Hetero-fertilization along with failed egg-sperm cell fusion reveals single fertilization involved in in vivo haploid induction in maize

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In vivo doubled-haploid technology is widely applied in commercial maize-breeding programs owing to its time-saving and cost-reducing features. The production of maize haploids during doubled-haploid breeding primarily depends on the use of the Stock6-derived haploid inducer lines. Although the gene underlying haploid induction, MTL/ZmPLA1/NLD, was cloned recently, how it functions is unknown. Hetero-fertilization can occur via a single fertilization, which provides an indirect way to investigate single-fertilization events by studying the hetero-fertilization phenomenon. We found that the hetero-fertilization rate increased significantly when female maize lines were first individually crossed with pollen from the inducer CAU5 in dual-pollination experiments for 4 h before the second pollination. We also examined ovule embryogenesis during haploid induction by confocal laser-scanning microscopy and found that, sometimes, a sperm cell fused only with a central cell, indicating that a single fertilization occurred during haploid induction. Consequently, we postulate that both single fertilizations and chromosome eliminations contribute to haploid production in maize. We also discuss a scheme for formation of hetero-fertilized and haploid kernels. Our results provide an efficient approach to identify hetero-fertilized kernels for research on interactions between embryos and endosperm.

Funding acknowledgement: the National Key Research and Development Program of China (2016YFD0101200), Modern Maize Industry Technology System (CARS-02-09)

P106

How to make maize seeds that look "not like dad": insights in double fertilization and prospects for novel breeding tools.

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Mixing male and female genetic information during sexual reproduction is considered as key to the evolutionary success of higher eukaryotes and is the basis of plant breeding. Sexual reproduction in flowering plants involves double fertilization, characterized by two separate fusion events between the male and female gametes. A maize line first reported in the 60s deviates from this classic pattern. Crosses using pollen from this so-called haploid inducer line, trigger the development of the egg cell into a haploid embryo with only the maternal genome, a process known as in vivo gynogenesis. Derivatives of this maize haploid inducer line have become the preferred tool of numerous maize breeding companies, because it can produce perfectly homozygous plants in only 2 generations instead of 5 to 8 in classical breeding schemes.
Our recent results (Gilles et al., EMBO J), together with two other simultaneous independent studies (Kelliher et al., Nature; and Liu et al., Molecular Plant), identified the major causal gene responsible for gynogenesis in maize. Our map based cloning restricted the QTL to a zone containing a single gene coding for a patatin-like phospholipase A, which was named *Not like Dad (NLD)* because haploid embryos do not have paternal contribution. In all surveyed haploid inducer lines *NLD* carries a 4 pb insertion leading to a predicted truncated protein. This frameshift mutation is responsible for haploid induction as complementation with wildtype *NLD* abolishes the haploid induction capacity. Translational NLD::citrine fusion protein likely localizes to the sperm cell plasma membrane. In Arabidopsis roots, the truncated protein is no longer localized to the plasma membrane, contrary to the wildtype NLD protein. In conclusion, an intact sperm-specific phospholipase is required for successful sexual reproduction and its targeted disruption may allow establishing powerful haploid breeding tools in numerous crops.

Gene / Gene Models described: *Not Like Dad (NLD)*; Zm00001d029412

P107

Identification and characterization of LINC complex proteins in *Zea mays* L.

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The LINC (Linker of Nucleoskeleton to Cytoskeleton) complex serves as an essential multi-protein structure that spans the nuclear envelope (NE). It connects the cytoplasm to the nucleoplasm and functions in both mechanical and signal transduction across the NE. In eukaryotes, the multiple functions of the LINC complex include the maintenance of nuclear shape, nuclear positioning within the cell, regulation of nuclear architecture, and chromosome dynamics during mitosis and meiosis. We have previously characterized the LINC-resident SUN domain proteins in meiotic prophase where they participate in tethering telomeres to the NE to ensure proper chromosome interactions needed for segregation. Here, we have identified and classified a total of 21 genes encoding candidate maize LINC complex proteins, grouped by their location in one of four cellular areas: the nucleoplasm (lamin-like NMCP/CRWN, and CRWN-interacting proteins); the inner nuclear membrane (SUN domain and NEAP proteins); the outer nuclear membrane (KASH and KASH-interacting proteins); and the cytoplasm (cytoskeleton-binding proteins). These genes were identified through bioinformatic screens and biochemical co-IP assays. Nuclear envelope localization of candidates was verified using heterologous fusion protein expression assays. FRAP-based assays further revealed ZmSUN2-dependent reduction of mobility for several of the KASH-FPs, indicative of KASH-SUN interactions. Transcriptome analysis revealed groups of tissue-specific co-expressed LINC genes. Genetic analysis revealed two phenotypes of interest, nuclear shape in root hair cells and stomatal complex development. Overall, we have identified genes encoding 9 KASH proteins (MLKp1-MLKp7, MLKt1, MLKt2), 3 NEAP proteins (NEAP1A, NEAP1B, and NEAP2), 5 SUN proteins (SUN1 - SUN5), and 4 CRWN or CRWN-binding proteins (NCH1, NCH2, KAKU4A and KAKU4B). These findings were used to develop a summary working model of the entire maize LINC complex, providing a framework for future studies of the plant nuclear envelope in a model crop species.

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P108

Identification of the translational landscape of Arabidopsis and Maize meiocytes

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Meiosis is essential in sexually reproducing organisms to maintain genome size from one generation to another. Moreover, meiosis is also the driving force for biodiversity, and hence, also the key for plant breeding. Recent studies in yeast have revealed that translational regulation is important to control protein abundance during meiosis. However in plants, as Arabidopsis and maize, meiotic gene regulation through translation is not known yet, although there are strong indications for pervasive translational control. To gain insights into potential translational regulation during meiosis in plants, we aim to identify the translatomes of dicoty ledonous and monocoty ledonous species, i.e. Arabidopsis and maize. To this end, we perform ribosome profiling experiments of isolated reproductive organs. A comparison with what is known in yeast and animals will then address the question whether the regulatory patterns and mechanisms are universally conserved and how translational regulation might have evolved.
Next to insights into gene regulation, these approaches also promise to reveal unknown meiotic regulators.

P109

Integrated analysis of protein abundance, transcript level, and tissue diversity to reveal developmental regulation of maize

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Integrated Analysis of Protein Abundance, Transcript Level, and Tissue Diversity to Reveal Developmental Regulation of MaizeThe differentiation and subsequent development of plant tissues or organs are tightly regulated at multiple levels, including the transcriptional, posttranscriptional, translational, and posttranslational levels. Transcriptomes define many of the tissue-specific gene expression patterns in maize, and some key genes and their regulatory networks have been established at the transcriptional level. In this study, the sequential window acquisition of all theoretical spectra-mass spectrometry technique was employed as a quantitative proteome assay of four representative maize tissues, and a set of high confidence proteins were identified. Integrated analysis of the proteome and transcriptome revealed that protein abundance was positively correlated with mRNA level with weak to moderate correlation coefficients, but the abundance of key proteins for function or architecture in a given tissue was closely tempo-spatially regulated at the transcription level. A subset of differentially expressed proteins, specifically tissue-specific proteins, were identified, e.g., reproductive structure and flower development-related proteins in tassel and ear, lipid and fatty acid biosynthetic process-related proteins in immature embryo, and inorganic substance and oxidation reduction responsive proteins in root, potentially revealing the physiology, morphology and function of each tissue. Furthermore, we found many new proteins in specific tissues that were highly correlated with their mRNA levels, in addition to known key factors. These proteome data provide new perspective for understanding many aspects of maize developmental biology.

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P110

Keeping Separate: mysterious boundaries and the grass leaf

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Boundary formation is a fundamental step in organ development. Boundaries allow organ separation, influence growth, polarity and shape, and act as the site of novel outgrowths (e.g grass ligules). Despite their importance, the gene regulatory networks (GRNs) responsible for boundary formation in different species are not well understood. This is especially true of the grasses, which have a unique, modular leaf structure with a wrapped base (sheath), a middle hinge region (ligule/auricle) and an upper flat region (blade). Grass leaf development requires the specification of three distinct boundary types: within-organ (e.g. ligule), inter-whorl (i.e. between successive leaves) and intra-whorl (i.e. between overlapping leaf margins). To discover how these boundaries are defined we are using two groups of maize mutants:
(1) Novel boundary mutants: *fused-leaf1 (fsl1)* and *fused-leaf2 (fsl2)* are intra- and inter-boundary mutants. We have identified chromosome positions for *fsl1* and *fsl2* using bulk-segregant analysis and chromosome walking, and surprisingly, neither mutation has implicated known boundary regulators. RNAseq of mature *fsl1* embryos further suggests that known organ boundary programs may not contribute to the *fsl1* phenotype. (2) Ligule mutants: Mutations in the grass specific transcription factors LIGULELESS1 (LG1) and LIGULELESS2 (LG2) cause ligule defects, but how they define the within-organ boundary is unknown. We are using yeast-2hybrid, CoIP, ChiPseq and RNAseq, to identify protein-protein interactions and directly regulated genes to elucidate the ligule GRN. Through this work we will build GRNs for intra-, inter- and within-organ boundaries in the grass leaf. By contrasting monocot leaf boundary programs with the dicot model *Arabidopsis*, we aim to determine how boundary specification is modulated in different species, possibly explaining novel morphology changes like the grass ligule.

Gene / Gene Models described: *LIGULELESS1, LIGULELESS2*; GRMZM2G036297, GRMZM2G060216
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P111

Kinetic and morphological tiller meristem development in domesticated and wild Setaria

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Setaria is a panicoid grass related to maize and sorghum. Lateral branches called tillers grow in grasses and commonly there are few to no tillers in domesticated grasses when compared to their wild ancestors. To explore tiller growth in wild Setaria (*S. viridis* line A10) and domesticated Setaria (*S. italica* lines yugu1 and B100), we measured axillary bud growth from 6 days after planting (DAP) up through 20DAP. We counted bud frequency in all three lines of Setaria and performed a statistical analysis on several tiller growth-related features, such as which leaves tillers originate from, the average number of tillers per inbred line, a comparison of primary versus secondary tillers, and a comparison of axillary versus auxiliary tillers. Lastly, we checked the growth in *S. viridis* and *S. italica* (B100 only) using scanning electron microscopy (SEM). Within B100, SEM photos showed a dynamic scale varying from fully mature axillary buds to no bud at all. We hope to further learn more about the mechanisms contributed to the variation of tiller meristematic development in Setaria.

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P112

Leveraging next-generation sequencing technology for rapid gene cloning

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Forward genetics remains a powerful mechanism for revealing the genes and gene networks that underpin organismal form and function. Forward genetic screens have the capacity both to reveal unexpected roles for known genes, and to reveal the functions of novel, uncharacterized genes. The size and complexity of the maize genome has made the identification of the genes underlying mutant phenotypes time consuming and challenging. However, the rise of next-generation sequencing (NGS) technology has allowed for the rapid identification of mutant genes. We developed a user-friendly NGS-based bulked segregant analysis pipeline that can be used to rapidly identify the lesions underlying mutant phenotypes in maize. We used this method to clone a number of genes with roles in flower and inflorescence development. We focus here, in particular, on a novel role we have uncovered for *ramosa3* (*ra3*) in the development of unisexual flowers. Our method offers an unbiased, cost-effective, simple strategy for rapid gene identification in maize.

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P113

MAC1 and *AM1* play critical but independent roles to regulate the mitosis-to-meiosis transition of pollen mother cells in maize

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In flowering plants, precursors of pollen mother cells (PMCs) first proliferate by mitosis, and then enter meiosis to produce haploid microspores. The mitosis-to-meiosis transition is a fundamental process for sexual reproduction; however, it remains largely unknown. I monitored this transition by labeling DNA replication in maize anthers and found that differentiated male germ cells first undergo asynchronous mitosis in an anther with a gradually decreased mitotic rate until reaching cell cycle quiescence in all PMCs. Next, the pre-meiotic S phase is initiated synchronously, followed by meiotic prophase I. The *multiple archesporial cells1* (*mac1*) mutant showed that the mitotic quiescence is absent and successive mitotic cell divisions result in extra PMCs. Surprisingly, without the mitotic quiescence in an *mac1* anther, the pre-meiotic S still occurs in some PMCs, whereas remaining PMCs undergo mitosis or arrest at interphase. In contrast, the *ameiotic1* (*am1*) mutant fails to enter pre-meiotic S phase. After a prolonged quiescence in *am1* PMCs, asynchronous mitosis resumes. The *mac1;am1* double mutant exhibited an additive phenotype. Taken together, these results indicate that MAC1 and AM1 are required for the mitotic quiescence and pre-meiotic S phase, respectively, in an independent manner.

Gene / Gene Models described: *Ameiotic1; Multiple archesporial cells 1*; Zm00001d013659; Zm00001d023681

P114

Maize genetics at the intersection of development and immunity

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The intersection between plant immunity and development is apparent in the phenotypes of many auto-immune mutants. However, the genetic mechanisms underlying this connection are not well established, especially in maize. The maize mutant *Liguleless narrow (Lgn-R)* has a severe developmental phenotype that is background dependent and temperature sensitive. In the B73 background, mutant plants are stunted with narrow leaves in cooler climes and completely dead when grown in hot field locations. However, in the Mo17 background the *Lgn-R* mutants are difficult to distinguish from non-mutant siblings in our cool fields and only develop severe mutant phenotypes in hot conditions. We have mapped the background dependent modifier of this mutant to *Sympathy for the ligule (Sol)*, a distant homolog of the Arabidopsis gene ENHANCED DISEASE RESISTANCE 4 (EDR4). The version of Sol found in Mo17 is capable of suppressing the mutant phenotype while the version found in B73 is incapable of this function. Furthermore, *Sol*, which normally has low expression levels, seems to be induced in the presence of certain PAMPs under particular conditions. To further delve into this system we generated large datasets, including an RNAseq and Phosphoproteome, for different combinations of the Lgn-R mutation and the modifier, Sol. We also saw signs of an induced immune response within these datasets. Therefore our data indicate that an immune response is potentially involved in the developmental defects found in our mutant and that the modifier may interact with this response differently in the diverse maize inbred lines. Further investigations into these genes should help to elucidate new aspects of both maize development and immunity.

Gene / Gene Models described: *Liguleless narrow (Lgn)*; GRMZM2G134382 (Lgn), GRMZM2G075262
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P115

Maize leaf primordia microdomains show genetic signatures of proximal-distal boundary patterning

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Understanding the factors that pattern organ boundaries is a central topic in plant biology. The maize leaf is a tractable developmental system as the ligule-auricle boundary between the distal blade and proximal sheath is delineated early in young leaf primordia. However, precisely when during leaf ontogeny are proximal-distal boundary (PDB) identities delimited remains an open question. We hypothesize that the competency to set up the PDB is founded in cellular/tissue microdomains in early-staged primordia long before morphological evidence of a PDB is observable in later-staged primordia. To identify genes that establish this "pre-PDB," we performed laser microdissection RNAseq (LM-RNAseq) across four contiguous proximal-distal microdomains beginning at the base of plastochron 4 (P4) leaf primordia, where the blade-sheath boundary is inconspicuous. Our analysis identified 1,045 differentially expressed genes across microdomains that partition into 25 nodes by self-organizing map clustering. Within our clusters, we found significant enrichment for transcription factor activity and hormone regulation gene ontologies. RNA *in situ* hybridization confirmed the spatiotemporal accumulation of candidate genes in developing primordia. We expanded our P4 mircodomain analysis to examine the expression pattern of genes in later-staged primordia, as well as and in *liguleless* (*lg*) mutants where the PDB is disrupted. Additional LM-RNAseq datasets from B73 (P7), *lg1* (P6) and *lg2* (P4, P6, and P7) mutants were combined to conduct a weighted gene co-expression network analysis (WGCNA) on a total of 61 LM-RNAseq libraries, which grouped 23,912 similarly expressed genes into co-expression modules. Our leaf microdomain-specific co-expression modules showed significant enrichment for known biological processes and identified leaf-specific expression modules. We found co-expression modules that contain LG1, LG2, and genes previously unknown to participate in leaf development. Overall, our findings reveal genetic signatures of a PDB in micordomains of early-staged primordia and highlight the power of analyzing multiple, high-resolution expression datasets to study leaf patterning.

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P116

Male-specific argonaute (MAGO) proteins are necessary for meiosis in maize

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Plants do not specify their germline until late in their life cycle. Hence, the plant germline is normally specified from terminally differentiated somatic cells though the precise mechanism(s) are unknown. We have found that male gametogenesis in maize is associated with the accumulation of distinct 21-nt phased small-interfering RNAs (phasiRNAs) generated by male-specific argonaute (MAGO) proteins. MAGO1 accumulates in the epidermis of pre-meiotic anthers while MAGO2 is found in developing meiocytes. We have found that MAGO proteins are required for meiocyte development as mutants display chromosomal defects and male infertility. Our data suggest that MAGO proteins play an important role in maize male fertility.

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P117

MicroRNAs targeting developmental transcription factors control maize leaf size by switching cell proliferation to cell expansion

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In maize, leaf size is determined both by cell division and cell expansion processes, which take place in three growth zones: meristem, elongation and mature zones, located linearly from leaf base to tip. Elucidating the genes controlling these processes is crucial for achieving increased leaf size. Recently microRNAs, endogenous non-coding small RNAs, have attracted attention as tools for alteration of gene expression. In order to gain insight into the microRNA regulatory networks behind the switching from cell proliferation to cell expansion in maize leaf, a miRNome analysis of 321 known maize microRNAs in the three distinct leaf growth zones was carried out. To induce growth retardation, ADA313 maize hybrid seedlings were subjected to low night temperatures, while a control group was grown for comperative studies. The fourth leaf of each seedling was monitored and the growth zones sampled for molecular analysis. The cold treatment caused a 19% reduction in leaf elongation rate resulting in a 26% decrease in final leaf size. To understand differences in the cell dynamics along the growth zones, lengths of cells are located in the same cell files were measured by DIC microscope and kinematic analysis of growth was performed. It was observed that the length and number of dividing cells in the meristem remained unchanged during cold treatment, but cell production declined by 30%. Genome wide analysis of microRNA displayed significantly differential expression along the growth zones for 204 out of 321 miRNAs. Cluster analysis identified 23 miRNAs as meristem- spesific. In silico target prediction suggested that these miRNAs target several transcription factors, and these interactions were validated by qRT-PCR. The findings clearly showed that many miRNAs play roles in controlling growth by switching between cell proliferation and elongation through targeting of transcription factors, suggesting that these miRNAs could be manipulated for crop improvement.

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P118

Modification of the expression of PIP2;5 plasma membrane aquaporin affects maize water relations and growth

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Aquaporins are highly regulated water channels controlling the water flow across cell membranes. We previously quantified the expression of plasma membrane intrinsic aquaporins (PIPs) in maize roots, leaves, stomatal complexes and in reproductive organs at both RNA and protein levels and showed that the expression was dependent on the organ developmental stage and environmental conditions.
In maize, PIP2;5 is the most highly expressed aquaporin in roots and its association with the presence of apoplastic barriers suggests a role in the regulation of radial water movement. Here, we investigated how deregulation of PIP2;5 expression affects water relations and plant growth using maize knockout (KO - W22 inbred) or overexpression (OE - B104 inbred) lines.
When growing in hydroponic culture, the hydraulic conductivity of cortex cells (Lpc) and the whole roots (Lpr) was lower in pip2;5 KO lines than in WT plants, as measured using a cell pressure probe or a hydraulic conductance flow meter (HCFM), respectively. While the Lpc was higher in PIP2;5 OE plants compared with the WT plants, no difference in the Lpr was recorded. In addition, the leaf hydraulic conductance (Kleaf) measured with the HCFM was higher in the PIP2;5 OE than in WT plants.
When growing in soil in well-watered conditions, no difference in the photosynthetic rate, stomatal conductance, and transpiration rate was observed between pip2;5 KO or PIP2;5 OE lines with their respective WT plants. On the other hand, drought stress treatment (three days without watering) significantly decreased these parameters in pip2;5 KO plants compared with the WT plants. We also found a less important decrease in the photosynthetic rate, stomatal conductance and transpiration rate in the PIP2;5 OE plants compared with B104 WT plants under drought stress condition. Altogether, these results indicate that PIP2;5 overexpression might be beneficial for plant growth under drought stress.

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P119

MOP1 regulates germline specification and gamete development in maize

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In angiosperms, germline initiates from a somatic subepidermal cell that acquires an archeosporial cell fate and then differentiates directly into the the megaspore mother cell (MMC). The MMC undergoes meiosis and from the resulting four megaspores, the three most apical degenerate while the surviving functional one will differentiate into the functional megaspore (FM). After three rounds of mitotic divisions, the FM will give rise to the mature megagametophyte or embryo sac that contains two gametes (the egg cell and the binucleated central cell) and accessory cells (two synergids and a cluster of antipodal cells). Specification of the germline, requires the orchestration of developmental programs and epigenetic mechanisms involved in the reprogramming and protection of the genome. Paramutation at the *b1* locus in maize is the most stable and penetrant example of transgenerational epigenetic inheritance known to date in nature. Mutations in the gene *mediator of paramutation 1* (*mop1*) prevents paramutation and induce pleiotropic developmental phenotypes including reduced height, feminization of tassels (tasselseed), alterations in flowering time, failure to develop ears and semi sterility. *mop1* encodes an RNA-DEPENDENT RNA POLYMERASE that regulates transposon activity as a component of the major small RNA-directed epigenetic regulatory pathway in plants known as the RNA-directed DNA methylation pathway (RdDM). In order to understand the role of RdDM during gamete development in maize, we performed a systematic cytological analysis during megasporogenesis and megagametogenesis and profiled global changes in gene expression in *mop1-1* mutant plants. We found that *mop1* is required for the specification of the female germline and identified a NAC transcription factor that when mutated leads to the phenocopy of the deffects observed during megasporogenesis in the *mop1-1* mutant.

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P120

Movement of premiotic phased small-interfering RNAs (phasiRNAs) is essential for male meiosis in maize

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Maize anthers accumulate a discrete group of 21- and 24-nt phased small-interfering RNAs (phasiRNAs). However, the precise function of these RNAs is currently unknown. The production of phasiRNAs is thought to be mediated by the slicing of long non-coding RNA precursors and initiated by discrete miRNAs. Pre-meiotic 21-nt phasiRNAs accumulate in the anther epidermis and are found in meiocytes later in development. We hypothesized that 21-nt phasiRNAs migrate from the epidermis to inner tissues during anther development. To test this hypothesis we have generated transgenic lines carrying viral proteins that sequester small non-coding RNAs in the anther epidermis. Our data suggest that movement of premiotic phasiRNAs is essential for anther meiosis in maize.

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P121

Natural variation in the molecular circuitry underlying cell type specification drives key plant architectural traits in maize

(submitted by Steffen Knauer <steffen.knauer@uni-tuebingen.de>)

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The shoot apical meristem (SAM), a specialized stem cell niche at the growing shoot tip, integrates developmental and environmental signals to direct the initiation and patterning of new organs such as leaves. Its activity throughout the plant's lifetime is tightly controlled. To gain insight into gene regulatory networks behind stem cell maintenance and organogenesis, we generated a high-resolution gene expression atlas of 10 distinct domains and cell types within the vegetative maize shoot apex using laser microdissection and RNA deep sequencing. We found that ~10% of all transcribed genes are differentially expressed across these tissue types, including a valuable collection of cell type specific genes. Interestingly, very few functional categories are enriched among the differentially expressed genes, which we show reflects prominent sub-functionalization within gene families. Through cluster analysis, we further identified expression signatures for the functional domains within the SAM, the stem cell harboring central zone (CZ), the peripheral zone (PZ), and organizing center (OC) located directly underneath the stem cells. Genes in these zones are in part conserved among maize and Arabidopsis, but also reveal remarkable differences and novel gene functions in maize. Moreover, we found that unique transcription factor (TF) signatures are predictive of meristematic and vascular cell fate. Analyses of TF binding sites within promoter regions of stem cell specific genes predicts a hierarchical network in which the combinatorial actions of diverse TF families underlie their spatially restrictive expression. Additionally, we demonstrate that KNOTTED1, a key meristem determinant, acts mainly by inhibiting organogenesis and differentiation within the SAM. Finally, we show that natural variation associated with important agronomical traits in GWAS, maps preferentially to genes showing domain specific patterns of expression or the TFs that drive this. Our findings thus indicate that gene regulatory networks acting in the SAM underlie adult plant architecture and important agronomical traits.

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P122

nop genes promote pollen tube growth in the maize male gametophyte

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Pollen tube growth is fundamental to plant reproduction. GRMZM2G372877 and GRMZM2G470666, tentatively designated *nop1* and *nop2*, were identified as highly-expressed in mature pollen relative to other maize tissues (Chettoor et al. 2014). These genes are orthologous to the rice *no pollen* (*Osnop*) gene (Jiang et al. 2005). Structurally, proteins encoded by the *nop* genes possess both calcium and phosphoinositide binding domains. Our current data support the hypothesis that *nop* genes have a role in pollen tube germination or growth, where they could help link calcium and/or phosphoinositides, which have important roles in pollen tubes, to other cellular processes.
Independent lines with transposable element insertions in exons of either *nop1* (*Ds*) or *nop2* (*Mu*) were found to be linked to gametophytically-expressed male-specific transmission defects (16.1% and 2.3% transmission, respectively). Derivative *nop1* alleles with non-frameshifted footprints were recovered in active *Ac* lines. The *nop1-d3* allele shows an increased transmission rate when compared to mutant *nop1* lines, proving that the transmission defect is due to loss of *nop1* function. A second mutant allele of *nop2* was acquired from the Dooner collection of *DsGFP*-tagged insertions (Li et al. 2013). This insertion confirmed a male transmission defect is associated with reduced *nop2* function.
Contrasting with the "no pollen" phenotype associated with a large deletion encompassing the *Osnop* gene, anthers and pollen grains have no visible phenotype in single *nop* mutant maize plants; nor was an obvious phenotype observed in pollen from *nop1::Ds*/+; *nop2::Mu*/+ plants. Using Inv9b to link *nop1::Ds* to the *wx1*$^{-}$ phenotype allowed us to differentiate *wx1*$^{-}$ (*nop1::Ds*-linked) and *Wx*$^{+}$ (*nop1*$^{+}$-linked) pollen in 1:1 segregating populations from heterozygous plants. When germinated *in vitro*, *nop1::Ds* pollen tubes were significantly shorter than wild-type pollen tubes, indicating that NOP1 promotes pollen tube growth and/or germination, and suggesting a possible explanation for the *nop1* male transmission defect.

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P123

Pursuing maize (*Zea mays*) tassel development by small RNA sequencing, transcriptomics, and proteomics

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During their life cycle land plants generate new organs from distinct meristems, containing a set of undifferentiated stem cells. In maize (*Zea mays*) tassel development, the inflorescence meristem (IM) initiates in parallel both branch meristems (BM) with an indeterminate fate and spikelet pair meristems (SPM). SPMs subsequently divide into two spikelet meristems (SM) and each of those produces two glumes and initiates the upper floral meristem (FM) and then converts itself into the lower FM. Florets contain four concentric whorls that are formed sequentially from the FM. First the palea and the lemma are initiated in the outer whorl, followed by the lodicule, then three stamens, and finally three carpels. In maize tassels all the carpels abort while the stamens differentiate into a supporting filament and the terminal anther. Within each anther 4 lobes are formed: these contain four distinct somatic cell types that are each required to support the central, pre-meiotic archesporial cells.
To determine the temporal progression of RNA, protein, and small RNA changes during tassel ontogeny, Agilent microarray transcriptomes, mass spectrometry proteomes, and small RNA-sequencing data were collected at 4 stages of early tassel development (0.5 cm, 1.0 cm, 1.5 cm, 2.0 cm). At the 0.5 cm and 1.0 cm stages tassels lack stamens, the 1.5 cm stage has stamens including anther primordia, and the 2.0 cm stage has anthers as large as 0.1 mm, prior to germinal specification within lobes. These data are the foundation to define meristem- and anther-specific genes for further functional study. Latest results from the data analysis and from gene-specific studies will be shown.

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P124

Redundant roles of two paralogous *INDETERMINATE DOMAIN (IDD)* transcription factors in maize development

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Functions of numerous genes controlling various aspects of maize growth and development have been elucidated, however comprehensive knowledge of gene regulatory networks that fine-tune key developmental processes remains limited. In maize, genome duplication resulted in functional redundancy among many gene pairs, thus masking phenotypic outputs in genetic screens. In this work, we investigate the functions of two paralogous *INDETERMINATE DOMAIN (IDD)* transcription factors (TFs), ZmIDD16 and ZmIDD18, in maize development. These two TFs share 88% amino acid identity. Based on co-expression network analyses of early tassel and ear development, *Zmidd16* was prioritized as a candidate gene in controlling inflorescence branching: i) its spatiotemporal expression across a panel of mutant backgrounds suggested a role in meristem determinacy, and ii) it was bound and positively modulated by the RAMOSA1 (RA1) TF, which functions to suppress branch outgrowth in maize inflorescences. *Zmidd16* and *Zmidd18* showed highly redundant gene expression profiles during inflorescence development, but *Zmidd18* was expressed consistently at lower levels and not bound by RA1. Preliminary *in situ* hybridization analysis showed that *Zmidd16* mRNAs localize to vascular tissues during early inflorescence development and in spikelet pair meristems, consistent with its regulation by RA1. To identify potential protein interacting partners of IDD16, we performed a yeast-two hybrid assay using a bait library derived from maize inflorescence primordia. Among high-confidence interactors identified were members of the *Networked* proteins, which are broadly thought to mediate actin interaction with plasma membranes. To test genetic perturbation of *Zmidd16* and/or *Zmidd18* on phenotype, we used CRISPR/Cas9-based gene editing to generate indels in coding sequences of both genes. T2 plants with homozygous edits in either paralog alone showed subtle effects on phenotype, while disruption of both genes resulted in pleiotropic developmental defects including severe dwarfism, increased tillering, short and broad leaves, sterile and branchless tassels, and anther-ear phenotypes.

Gene / Gene Models described: *IDD16, IDD18*; GRMZM2G074032, GRMZM2G465595
Funding acknowledgement: National Science Foundation (NSF)

P125

Relative abundance of Proteins to Multiple stresses suggests Cross-Tolerance Mechanism in Soybean

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Water stress (WS) and high temperatures (HS) have a negative effect on soybean crop productivity. During WS, soybean plants opt for survival through ion homeostasis and the conformations of proteins are perturbed as plant cells lose water, while HS leads to difficulties in flowering and fruiting. Proteomic studies were conducted to obtain insight into the effects of WS and HS on molecular and cellular functions. Two soybean cultivars (A: Slow wilting and high yielding; and B: High protein with moderate yield potential) were exposed to different heat and water stress conditions in plant growth chambers. Changes in the leaf protein composition were studied using 2-DE complemented with MALDI-TOF mass spectrometry. Thirty-nine proteins were differentially expressed in response to WS and HS in both cultivars. Gene ontology analysis revealed majority of the functional categories including photosynthesis, metabolism, transport, stress and defense, and glycolysis. Expression of proteins was largely affected to WS in Cultivar A, while HS affected their expression in cultivar B suggesting genetic variation for stress tolerance. Combined abiotic stresses (WS+HS) equally affected both cultivars. RT-PCR studies and enzyme assays of selected genes suggest a positive correlation with protein expression among the cultivars. Proteins involved in metabolism, glycolysis, photosynthesis, such as HSPs, enolase, rubisco activase, were over expressed during WS+HS stress in both cultivars. Based on protein interaction studies, we hypothesize the plant's development of cross-stress tolerance.

P126

Responses to hypoxia and endoplasmic reticulum stress: relationships with vitreousness of maize endosperm

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Endosperm vitreousness is an important quality trait of maize crops. To delineate the mechanisms controlling the formation of endosperm texture, i.e. compactness of the starch/protein matrix, most studies have been focused on opaque/floury mutants, never on conventional maize crops. Therefore, we analyzed protein and starch depositions in the floury and vitreous regions of mature endosperms of conventional flint and dent maize inbred lines. We disclosed biochemical gradients, with a continuous decrease of protein contents and an increase of starch crystallinity (related to an increase of amylopectin/amylose ratio) from the periphery (subaleurone region) to the center of endosperm. To grasp these gradients, we analyzed the transcriptome and specific metabolites of developing central and peripheral endosperms (at 15 and 20DPA) that will become, later, the floury and vitreous regions of mature seeds, respectively. The results revealed clearly that the formation of endosperm vitreousness is associated with significant differences in the responses to two closely linked stress phenomena, hypoxia and endoplasmic reticulum stress; hypoxia being probably the major stress affecting endosperm development. Indeed, transcriptomic and metabolomic data indicated a strong regulation of energy metabolism in developing endosperm. Genes involved in glycolysis and tricarboxylic acid cycle are up-regulated in the periphery, while genes involved in alanine, sorbitol and fermentative metabolisms are up-regulated in the center of endosperm. Besides hypoxia, the massive synthesis of proteins (mainly storage proteins, i.e. zeins) in the endoplasmic reticulum elicits unfolded protein responses (UPR), as indicated by the splicing of bZip60 transcription factor. UPR was differentially regulated between the center and periphery of the endosperms. Taking together, these results suggested that regulation of energy metabolism and UPR allow the production of ATP needed for protein synthesis and their folding in the endoplasmic reticulum, respectively. Spatial regulation of these mechanisms within endosperm probably drives the compositional gradient, governing hence maize vitreousness

Funding acknowledgement: Fond Unique Interministériel

P127

RMR12 is a CHD3 nucleosome remodeler required for maintaining paramutations and normal development

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In maize, paramutations result in meiotically heritable changes in the regulation of certain alleles of *purple plant1* [1], a gene encoding a transcription factor required for anthocyanin production[2]. A strongly expressed *Pl1-Rhoades* allele is suppressed in trans when combined with a transcriptionally and post-transcriptionally repressed *Pl1-Rhoades* allele, and both alleles are transmitted in a repressed (denoted *Pl′*) state. At least sixteen loci whose functions are *required to maintain repression (rmr)* of *Pl′* have been identified by ethyl methanesulfonate mutagenesis[3]. All known RMR proteins mediate 24 nucleotide (24nt) RNA biogenesis[4, 5, 6, 7, 8], and four are putative orthologs of *Arabidopsis* proteins central to an RNA-directed DNA Methylation (RdDM) pathway facilitating repressive chromatin modifications. Here we describe four recessive alleles defining the *rmr12* locus. Unlike other *rmr*-type mutations found to date[4, 5, 7, 8, 9, 10], *rmr12* mutants display a unique set of defects, including male gametophyte dysfunction, that highlight a novel mechanistic connection between paramutation and developmental gene control. We used positional cloning to discover that *rmr12* encodes a Chromodomain Helicase-DNA Binding3 (CHD3) protein whose presumed *Arabidopsis* ortholog, PICKLE, alters nucleosome positions in vitro[11] and affects both development and chromatin modifications specified by RdDM[12, 13]. Maize CHD3 represents the first RMR protein not having a predicted role in 24nt RNA biogenesis and thus might facilitate paramutations by converting 24nt RNA effectors into meiotically-heritable nucleosome profiles.

1. Hollick *et al*. 1995 *Genetics* **141**, 709. | 2. Cone *et al*. 1993 *Plant Cell* **5**, 1795 | 3. Hollick and Chandler 2001 *Genetics* **157**, 369 | 4. Erhard *et al*. 2009 *Science* **323**, 1201 | 5. Hale *et al*. 2007 *PLoS Biol*. **5**, 2156 | 6. Nobuta *et al*. 2008 *PNAS* **105**, 14958 | 7. Stonaker *et al*. 2009 *PLoS Genet*. **5**, e1000706. | 8. Barbour *et al*. 2012 *Plant Cell* **24**, 1761 | 9. Dorweiler *et al*. 2000 *Plant Cell* **12**, 2101. | 10. Parkinson *et al*. 2007 *Dev. Biol*. **308**, 462. | 11. Ho *et al*. 2013 *Biochim. Biophys. Acta* **1829**, 199 | 12. Ogas *et al*. 1997 *Science* **277**, 91 | 13. Yang *et al*. 2017 *Genome Biol*. **18**, 103

Gene / Gene Models described: *rmr12/chd3a*; Zm00001d045109
Funding acknowledgement: National Science Foundation (NSF), United States Department of Agriculture (USDA), Syngenta

P128

Role of introns in the correct expression of the meiotic cyclin SOLO DANCERS (SDS) of maize in A. thaliana

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Meiosis, in contrast to mitosis, halves the nuclear DNA content. This is important for sexual reproduction to maintain genome size in the offspring. Moreover, meiosis accomplishes recombination between the parental homologous chromosomes leading to new allelic combinations in the offspring. Despite the differences between meiosis and mitosis, the progression through both cell division events appears to be regulated by the same or highly related regulators, such as cyclin-dependent kinases, cyclins and the anaphase-promoting complex/cyclosome, etc. One of the meiosis specific cyclins is called SOLO DANCERS (SDS) and has been described before in A. thaliana. There, SDS is expressed specifically in male meiotic cells where it functions in homolog synapsis, recombination and bivalent formation. While putative SDS homologs in Zea mays have already been discovered, their actual biochemical function in the cell cycle remains unknown. Due to its agronomical relevance the regulation of meiosis in maize in general as well as the recombinational process in particular should be studied. In this study rescue experiments with the genomic sequence of one of the putative orthologues, ZmSDS57, were conducted in order to complement sds deficient Arabidopsis thaliana mutants. In preliminary experiments it was found that this gene driven by the AtSDS promoter is not able to rescue the mutant, whereas the same construct with the genomic ORF of AtSDS does. On the other hand, the cDNA ORF of AtSDS did not show any sign of expression either. These observations can lead to the idea that the introns harbored in the genomic SDS sequence can have an important role at the expression level. In order to confirm this, several constructs exchanging and deleting introns have been designed. It is known that the first two introns are more likely to contain regulatory elements. Therefore, the focus was put on that region of the sequence.

Gene / Gene Models described: *solo dancers (SDS)*; GRMZM2G093157

P129

Search for genetic modifiers of *knotted1*

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Over two decades ago the maize gene *knotted1* (*kn1*) was cloned, marking the discovery of the first homeodomain-containing transcription factor in plants and a key regulator of shoot apical meristem (SAM) maintenance. The KNOTTED1 protein is strongly expressed in the SAM as well as the subtending rib and inflorescence meristems where it binds and modulates the expression of hundreds of genes. Despite its known contribution to SAM maintenance, the penetrance of the shoot termination phenotype caused by the loss-of-function *kn1-e1* allele varies dramatically between inbred lines. While B73 plants homozygous for *kn1-e1* exhibit a phenotypic penetrance of less than 5%, W23 plants are severely affected, with 95% of individuals failing to maintain a SAM. Penetrance of *kn1-e1* is thought to negatively correlate with SAM size.
We sought to determine the genetic basis for this difference in phenotypic penetrance by performing laser capture microdissection followed by RNA-Seq on the SAMs of normal B73, *kn1-e1* B73, and normal W23 plants. Few genes were differentially expressed between normal B73 and *kn1-e1* B73 SAMs, suggesting that the transcriptional network controlling SAM maintenance is robust to loss of *kn1* function in this line. Meanwhile, the SAMs of W23 exhibited differential expression of several thousand genes compared to those in B73. Included are genes bound and modulated by *kn1* as well as the closely related paralog *gnarley1* (*gn1*), which was significantly upregulated in B73 compared to W23. In addition, in a previously generated dataset, *gn1* was more highly expressed in SAMs of inbreds with large and medium compared to small SAM sizes. We therefore hypothesize that increased expression of *gn1* in the SAM of B73 compensates for loss of *kn1* function, which will be tested in future reverse genetic analysis.

Gene / Gene Models described: *knotted1, gnarley1*; GRMZM2G017087, GRMZM2G452178
Funding acknowledgement: National Science Foundation (NSF)

P130

Sex and violence: the classic maize mutant *Tasselseed5* is encoded by a wound-responsive JA-inactivating enzyme

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Notable both for a long history and genotype-dependent expression, *Tasselseed5 (Ts5)* mutants have ectopic silks in the tassel and ear row defects from failed lower floret abortion. Maize flowers of both the tassel and ear are initially perfect but become staminant or pistillate by selective abortion of carpels in tassel florets and stamen in ear florets. Using a combination of fine-mapping and transcriptomics we found that *Ts5* encodes a CYP94B3, known to convert the bioactive jasmonate, JA-Ile, to less active 12OH-JA-Ile, as part of normal recovery from wounding or herbivory. Our RNAseq analysis identified over 240 DE genes in 1cm tassels with putative functions that are consistent with the predicted enzymatic role of CYP94B3. The *Ts5* gene had a logFC of 11.7 strongly implicating its ectopic upregulation as causal to its observed tassel feminization. *Ts5* tassel phenotypes are suppressed by exongenous JA application and metabolite profiling for jasmonate derivitives in both developing tassels and wounded leaves, show enhanced catabolysis of JA in *Ts5* mutants. Thus, misexpression of *Ts5* in inflorescences destroys monoecy by breaking down JA -- which is required for carpel abortion in tassels and lower floret abortion in ears. Heterologous overexpression in both a dicot (Arabidopsis) and monocot with perfect flowers (*Brachypodium distachyon* (L.) P.Beauv.) uncovered known and novel JA-deficient phenotypes. To explore natural modifiers of the *Ts5* phenotype, we performed a QTL analysis leveraging the fact that *Ts5* is completely feminized in Mo17 and nearly fully suppressed in B73. One of 10 high-confidence QTL interval contains *tasselseed2* with which *Ts5* displays epistasis (Irish et al. (1994) Dev Genet 15: 155–171), is also wound-inducible, and is misexpressed in *Ts5* .

Gene / Gene Models described: *Ts5*; Zm00001d049201
Funding acknowledgement: National Science Foundation (NSF), United States Department of Agriculture (USDA)

P131

Single Cell Transcriptomic Analysis of the Developing Maize Embryo

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Single cell RNA-Seq (scRNA-Seq) has emerged as a technology to facilitate the high-throughput transcriptomic analysis of individual cells. Numerous scRNA-Seq studies are reported in animals; however, the technology has seen limited use in plants. Here we present preliminary single cell transcriptomic data generated from maize embryos using the 10X Genomics Chromium platform. The maize embryo was selected as a study system owing to its diversity of cell and tissue types, including the embryonic shoot and root apical meristems (SAM and RAM, respectively). We aim to use this technology in combination with existing *in situ* hybridization and RNA-Seq data to examine the contributions of cell-type heterogeneity, signaling, and differentiation programs to SAM patterning and development, and to resolve single-cell gene co-expression networks.
Analysis of the dataset identifies embryo-specific marker genes, including previously-described lowly-expressed genes and transcripts accumulating in small cell populations. Expression analysis of characterized markers enables assignment of cells back to their spatial positions in the embryo. A preponderance of cells expressing epidermal cell marker genes were identified, suggesting biases in cell isolation. Nonetheless, marker gene expression analysis and pseudotemporal ordering of cells using manifold learning reveals potentially pertinent developmental biology. These findings include the identification of *yab9* as a potential regulator of scutellum development and dynamic changes in cell cycle genes over pseudotime that may be correlated with epidermal cell proliferation rate and differentiation.
Future work will attempt to circumvent the unique challenges of unbiased cell-type isolation in plants by using nuclei as a source of RNA for transcriptomic profiling, and utilizing higher cell/nuclei populations to enhance statistical power.

Gene / Gene Models described: *yabby14, yabby9*; GRMZM2G005353, GRMZM2G074543
Funding acknowledgement: National Science Foundation (NSF)

P132

The genetic control of a new tassel seed mutant in maize

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Unisexual maize flowers originate through selective abortion of female primordia in the tassel and of male stamens in the ear from bisexual inflorescences. Tassel seed mutations are known to alter the usual sex fate allowing carpel survival in the male inflorescence. Objective of the present research is to describe and map a novel tassel seed phenotype shown by an inbred line, Rig7, identified among a set of lines derived from in vitro regeneration. Genetic mapping was carried out using a B73 x Rig7 F_2 population (genotyped with 15K SNP array) and by SNP-based bulk segregant analysis using two additional populations (BC_1 and F_2). Both approaches clearly indicated that the tassel seed phenotype is under the control of two loci mapping on chromosomes 2 and 6. The locus on chromosome 2 showed remarkable dominant epistasis on the second locus and was mapped near the known tassel seed gene *ts1*, in the pericentromeric region. The locus on chromosome 6 was mapped to a 130-kb region which included three genes based on B73 genome annotation. Candidate genes are being tested by reverse genetics, comparison of allele sequences and by analysis of different hormones concentration in young tassels.

P133

The role of boron in vegetative and reproductive development in maize

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Boron deficiency is a common abiotic stress negatively affecting crop yield worldwide. We have identified the *tasselless1* (*tls1*) gene as the co-orthologue of the Arabidopsis boron importer NIP5;1. Since the *tls1* mutant cannot actively take up boron out of the soil, it is inherently boron deficient. By using this mutant, we found that one of the earliest symptoms of boron deficiency is a reduction in meristem size. Depending on the boron availability in the soil, this leads to vegetative and/or reproductive defects, which can all be rescued by boron supplementation. In order to understand what causes the reduction in meristem size in *tls1*, we are studying the involvement of boron in meristem maintenance pathways as well as in hormone pathways regulated by cytokinin and auxin. We are analyzing double mutants between *tls1* and known meristem pathway mutants and with mutants involved in auxin/cytokinin biosynthesis and signaling. These analyses are combined with confocal and fluorescence microscopy of marker genes such as ZmWUSCHEL:RFP.
We also found, that the tassel phenotype of *tls1* can be rescued by additional light in the greenhouse. We found this effect to be correlated with increased transpiration, which indicates that passive boron transport is a major boron source in maize, even under boron deficiency conditions. Our studies will aid in understanding the role of boron in plant development and will ultimately lead to the development of higher yielding plants in marginal soils.

Funding acknowledgement: United States Department of Agriculture (USDA)

P134

The role of CT2 in maize internode development

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Heterotrimeric G proteins are membrane-associated molecular switches and are composed of the three subunits Gα, -β and -γ. They transduce extracellular signals to induce specific cellular responses by activating downstream effectors and are involved in a wide range of growth and developmental processes in animals and plants.
In maize COMPACT PLANT2 (CT2) was identified as the α-subunit. Compared to wild type the shoot apical meristem of *ct2* mutant plants is enlarged and internodes are shortened, indicating that CT2 influences cell proliferation and elongation. These processes are orchestrated by the reorganization of microtubules. Although CT2 does not bind directly to microtubules *in vitro*, the Gα subunit might influence their reorganization *in vivo* via interacting proteins which bind both to microtubules and CT2. In initial IP/MS experiments with the CT2-YFP reporter line we identified the Phospholipase Dα5 (ZmPLDα5) as a potential CT2 interacting protein. Phospholipases have been shown to influence microtubule reorganization in *Arabidopsis* and additionally AtPLDα1 physically interacts with Gα to accelerate GTP hydrolysis *in vitro*, indicating that AtPLDα1 is a GTPase accelerating protein (GAP). In maize no GAPs are known so far, so these proteins could illuminate the regulatory network of maize G protein signaling.
We investigated the possibility of the AtPLDα1 homolog ZmPLDα2 being the missing link between microtubule reorganization and G protein signaling as well as being the missing GAP in maize. *Förster Resonance Energy Transfer* (FRET) and co-immunoprecipitation (Co-IP) data indicate an interaction at the plasma membrane of transiently transformed tobacco leaf cells. Additionally Loss-Of-Function (LOF) lines were identified to analyze their genetic interaction.

Gene / Gene Models described: *compact plant2, phospholipase D2*; GRMZM2G064732, GRMZM2G061969
Funding acknowledgement: German Research Foundation (DFG)

P135

The role of maize mutant *Suppressor of sessile spikelets 2* (*Sos2*) in meristem maintenance

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Plant development is driven by a group of undifferentiated stem cells, called meristems. During maize reproductive development, the shoot apical meristem (SAM), responsible for above ground growth, transitions into an inflorescence meristem (IM) which then produces a series of meristems along the periphery to give rise to the male reproductive structure called the tassel. The female reproductive structures, or ears, arise similarly from elongation of axillary meristems half-way down the stem. This developmental progression requires a fine balance of stem cell proliferation and differentiation, referred to as meristem maintenance. My research focuses on the semi-dominant maize mutant *Suppressor of sessile spikelets 2* (*Sos2*), which has defects in meristem maintenance in all above ground meristem types, resulting in altered meristem size and timing of termination. *Sos2* heterozygous plants have decreased branching in the tassel and suppression of spikelets, the short flower-bearing branches, in the tassel and the ear. If a tassel is formed in *Sos2* homozygotes, it is short and bifurcated and ears are small and ball shaped. To determine the *Sos2* gene function, I have analyzed double mutants of *Sos2* with *CLAVATA* pathway mutants, previously shown to play a role in meristem maintenance. In addition, fluorescent microscopy of *ZmWUSCHEL:RFP* transgenic marker lines was used to characterize the stem cell niche of *Sos2* mutants. The region was *Sos2* maps on chromosome 10 has been sequenced and dosage analysis has been performed to determine the gene responsible for the *Sos2* phenotype and the type of dominance seen in *Sos2* populations. Our results indicate that *Sos2* plays a fundamental role in meristem maintenance throughout plant development.

Funding acknowledgement: National Science Foundation (NSF)

P136

The role of ZmSCR1 and ZmSCR1h during maize leaf development

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In most cases, the C_4 photosynthetic pathway is underpinned by characteristic Kranz anatomy, with concentric wreaths of bundle sheath (BS) and mesophyll (M) cells surrounding closely spaced veins. It has been hypothesised that the SHORTROOT/SCARECROW (SHR/SCR) transcription factors act to regulate Kranz anatomy development in maize. In support of this, *Zmscr1* and *Zmshr1* mutants show perturbations in Kranz formation. However, the phenotype exhibited in *Zmscr1* mutants is relatively subtle, with many vascular bundles appearing to develop normally. Here, we show that ZmSCR1 functions redundantly with the closely related homeolog ZmSCR1h. Double mutant *Zmscr1-1; Zmscr1h-1* plants exhibit a reduced growth phenotype not seen in either of the single mutants, with drooping leaves caused by incomplete midrib extension. Furthermore, *Zmscr1-1; Zmscr1h-1* plants exhibit more severe perturbations in BS and M cell patterning than previously found in *Zmscr1* single mutants, as well as clear alterations in vein order formation. Expression of maize *SHR* orthologs do not appear to be altered in the *Zmscr1-1; Zmscr1h-1* background. We hypothesise that many of the observed phenotypic perturbations may be caused by increased SHR movement in the absence of SCR. To test this, we have generated SHR and SCR antibodies to enable protein accumulation to be compared. This study has provided insight into how the SCR/SHR pathway acts to regulate Kranz anatomy development in maize.

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P137

The *scarecrow* mutation enhances auxin-related leaf and inflorescence defects in maize

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Decades of research have underscored the role of auxin in the morphogenesis of plant organs. Auxin biosynthesis and transport, have been known to steer early morphogenic events while numerous auxin signals and responses have also been identified to fine-tune developmental processes from cell division to tissue differentiation. On the other hand, non-hormone players such as transcription factors have been recognized as key components of various developmental pathways. The connection between auxin and non-hormone regulators of plant development still needs to be understood in detail. The transcription factor SCARECROW (SCR) was identified as a key regulator of endodermis development in roots and more recently as a developmental switch for bundle sheath differentiation in maize. The endodermis/bundle sheath, a high-capacity auxin conduit, has been recognized as a critical spatial hub for regulation of root and shoot development. Here, we report genetic evidence of possible connections between auxin and *ZmSCR* during leaf and inflorescence development in maize. Double mutant analyses reveal enhancement of phenotypes attributed to defective auxin biosynthesis and transport. These observations indicate a possible genetic interaction between auxin and a tissue-specific transcription factor and demonstrate other ways in which auxin shapes organ development in maize.

Funding acknowledgement: The Fulbright Program

P138

Tissue- and cell-specific multi-omics analyses define a key molecular pathway of lateral root initiation and its interaction with arbuscular mycorrhizal fungi in maize

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Heterogeneity of xylem- and phloem-pole pericycle cells determines their competence for lateral root initiation. Outgrowth of lateral roots from competent phloem-pole pericycle cells allows them to forage for soil resources. Arbuscular mycorrhizal (AM) fungi play an important role in triggering the developmental program of their host roots. The present study aims to decipher the mechanisms of post-embryonic lateral root initiation and their interaction with AM fungi in maize.
Systemic histological and histochemical analyses of the lateral root defective mutants *lateral rootless 1* (*lrt1*) and *rootless with undetectable meristem 1* (*rum1*), revealed excessive cell wall lignification of phloem-pole pericycle cells in these mutants. We surveyed the transcriptome signatures of xylem- and phloem-pole pericycle cells of these mutants isolated during different stages of lateral root initiation by laser capture microdissection (LCM)-based RNA-seq. We demonstrated that cell wall biogenesis and organization are key processes controlling pericycle cell competence.
We further determined 12 inbred lines with disparate lateral root initiation frequency from the intermated B73-by-Mo17 (IBM) population. In general, lines with few lateral (FL) roots displayed higher cell wall lignification in pericycle cells. Comparative transcriptome profiling of manually dissected steles revealed that genes involved in ubiquitination and phenylpropanoid biosynthesis were exclusively enriched in FL lines.
To explore the role of AM fungi on lateral root initiation, we demonstrated that AM fungi (*Rhizophagus irregularis*) exclusively induce lateral root formation in FL lines and *lrt1*. Tissue-specific metabolome (GC-MS) and proteome (iTRAQ) analyses of *lrt1*, demonstrated that AM fungi induce lateral root formation by interfering with biosynthetesis of phenylpropanoid-related compounds. Moreover, the transcriptomes of isolated phloem-pole pericycle cells from AM-treated and non-AM-treated *lrt1* plants showed diverse regulation of cell wall-related genes. Taken together, these results highlight a novel role of phenylpropanoid-related cell wall biosynthesis in lateral root initiation by interacting with AM fungi in maize.

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P139

Towards a comprehensive understanding of genetic architecture underlying senescence

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Senescence is a very important agronomic trait due to its close association with photosynthetic assimilation, partitioning of photoassimilate among reproductive and vegetative organs, and the ability of the plant to cope with biotic and abiotic stress. Senescence represents a major shift in plant developmental program and is, therefore, under a highly complex genetic regulation. Among a multitude of internal and external factors, senescence is also regulated by source-sink cross-talk. For instance, we and others have shown that removal of primary sink leads to disruption of carbohydrate partitioning among source and sinks, and triggers source-sink regulated senescence (SSRS). We are using a systems genetics approach to dissect the genetic, physiological, and metabolic determinants of natural senescence and SSRS. Through extensive multi-location and multi-year phenotyping a diversity panel and a bi-parental RIL population, we have identified very strong associations and QTL underlying natural senescence and SSRS. Further analyses of these associations revealed several novel genes underlying natural senescence and SSRS. QTL underlying SSRS were confirmed by near-isogenic lines (NILs) and the QTL introgressions in NILs were reduced in size through backcrossing. Remarkably, we identified several candidate genes underlying SSRS, however, none of these have been implicated in carbohydrate partitioning, thus suggesting mechanistically unique regulation of SSRS. We are currently performing transcriptomic and metabolic analyses to corroborate the associations and QTL. Experiments are also undergoing to validate and further understand the role of candidate genes in natural senescence and SSRS, and those results will be presented.

Funding acknowledgement: Department of Energy (DOE)

P140

Towards live imaging of meiosis in maize

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Meiosis is a specialized cell division, which reduces the genome by half through two consecutive chromosome separation events. Meiosis is key to biological diversity since homologous chromosomes exchange DNA fragments in the process of meiotic recombination, giving rise to novel combination of parental alleles. Due to this function, meiosis is also central to plant breeding. Despite of its importance, many crucial steps in meiosis are not well understood. So far, most studies of meiosis in plants have relied on classical genetic analyses and cytological observations of fixed chromosome spreads. Although important and informative, these studies have limits in accurately examining the dynamics of meiotic processes and investigating temporal and spatial aspects. Here, we present our set up of an efficient live imaging system for maize meiosis that is based on a previously established protocol in our team to follow meiosis in Arabidopsis.
The method relies on transgenic lines producing fluorescently labeled proteins that highlight hallmarks of meiosis. We selected different live imaging markers, by which specific aspects of meiosis are visualized. These include CENH3 (a centromeric histone variant) as chromosome marker as well as DSY2 and ZYP1 as elements of the synaptonemal complex to follow chromosome movement and pairing. Furthermore, AM1 and SDS as markers to monitor entrance and progression through meiotic stages and COM1 and MUS81 to visualize recombination events. By themselves and especially in combination, these different reporter lines will be a useful tool to reveal the dynamics of meiotic chromosome behavior in vivo and define a time course of meiosis in maize.

Gene / Gene Models described: *Am1; ASY3; CENH3; MUS81; SDS; COM1; Zyp1*; GRMZM5G883855; LOC103626703; GRMZM2G158526; GRMZM5G822970; GRMZM2G093157; GRMZM2G076617; GRMZM2G143590

P141

Transcriptomic characterization of male sexual reproduction in maize

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Sexual reproduction in plants involves developmental regulation of key cellular processes (e.g., pollen tube growth, cell-cell signaling, fertilization). Recent results demonstrate that plant genomes undergo large scale alteration of gene expression and epigenetic modifications as plants undergo meiosis to produce haploid gametophytes for the next generation. We have undertaken a transcriptomic study of male sexual development in maize to investigate these regulatory events.
We developed methods to isolate and sequence mRNA and small RNA from the same tissue sample at four developmental stages, with four biological replicates for each stage: tassel primordia, microspores, mature pollen, and sperm cells. PCA analysis of the datasets indicate that replicates from each tissue have low variance, enabling statistical confirmation of any observed differential expression of genes/transposable elements between tissues. We additionally show that our mature pollen sequencing datasets resemble previously sequenced high-quality datasets. Gene ontogeny analysis of mRNA transcripts highlights contrasting cellular processes in mature pollen, consistent with the distinct roles of the sperm cells and the vegetative cell in reproduction. To better assess dynamic transposable element regulation in maize male reproductive tissues, we developed a bioinformatic tool to easily visualize and identify differentially expressed transposable elements in these samples.
Intriguingly, genes that are highly-expressed in either mature pollen or sperm cells are associated with fewer transposable element insertions in exons in sequenced populations (UniformMu, Photosynthetic Mutant Library), relative to highly-expressed seedling genes. This is consistent with the idea that deleterious mutations in genes important for either pollen or sperm cell function are subject to relatively higher purifying selection, likely due to the haploid nature of these stages.

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P142

Transient interaction analysis of the *Zea mays* (L.) CT2 and PLDα1 proteins in *Nicotiana benthamiana* (L.)

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Heterotrimeric G protein signaling is involved in a variety of growth and developmental processes in animals and plants. These membrane-associated complexes consist of the three subunits Gα, -β and -γ. The number of subunits differs from animals to plants. Plants usually have one canonical α-, one β- and three to six γ-subunits. In maize COMPACT PLANT2 (CT2) has been identified as the α subunit of the heterotrimeric complex.
The regulation of G protein signaling differs in plants as well. There are no G Protein Coupled Receptors (GPCRs) in plants. In addition, maize, like many other grasses, lacks the REGULATOR OF G PROTEIN SIGNALING (RGS).
In *Arabidopsis* it has been demonstrated that Phospholipase AtPLDα1 interacts with Gα (GPA1) via a conserved DRY motif that is also required for the interaction of multiple GPCRs with Gα subunits in animal systems. Furthermore it has been shown that AtPLDα1 can act as a GTPase accelerating protein (GAP) on AtGPA1 *in vitro*.
As we identified maize PHOSPHOLIPASE Dα5 (ZmPLDα5) in previous CT2-YFP IP/MS experiments as a CT2 interacting protein, we continued to investigate the interaction between CT2 and ZmPLDα1, the likely maize ortholog of AtPLDα1 using *Förster Resonance Energy Transfer* (FRET) and co-immunoprecipitation (Co-IP) experiments. Our results indicate an interaction of ZmPLDα1 and CT2 at the plasma membrane, which is dependent of the DRY motif. This interaction might expand the regulatory network of maize G protein signaling.

Gene / Gene Models described: *compact plant2, phospholipase D1*; GRMZM2G064732, GRMZM2G054559
Funding acknowledgement: German Research Foundation (DFG)

P143

AFD1 and DSY2 are both required for chromosomal localization of ASY1 and meiotic double-strand break formation in maize

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Meiosis is a specific type of cell division required for generating haploid gametes. It contains single round of DNA replication, followed by two rounds of chromosome segregation. Meiotic recombination initiated by double-strand breaks (DSBs) are essential for homologous pairing and the proper segregation of homologues at metaphase I. Synapsis, a process of synaptonemal complex (SC) formation between newly paired homologous chromosomes, regulates meiotic recombination. Despite the importance of SC proteins in meiosis, relatively little is known about their genetics and molecular biology in plants. In maize, the known SC proteins include axial element components ASY1 and AFD1, and the central element component ZYP1. Previously, we had identified DESYNAPTIC2 (DSY2) as an axial element protein and showed that DSY2 is required for normal level of DSBs and SC formation. In this study, we found that proper localization of DSY2 and ASY1 depends on AFD1. In contrast to a linear pattern of both DSY2 and ASY1 in the wild-type, DSY2 and ASY1 form short stretches in the *afd1-1* mutant. On the other hand, ASY1 shows a weaker but linear signal in *dsy2*, indicating that DSY2 is required for the proper recruitment of ASY1. Interaction between DSY2 and ASY1 was confirmed by both yeast-two hybrid and immunoprecipitations of anti-DSY2 and anti-ASY1. The double mutant lacking both *Dsy2* and *Afd1* shows a synergistic phenotype that ASY1 signals appear diffused and unable to load on chromosome. In addition, the number of γH2AX and RAD51 foci is totally abolished in *dsy2 afd1* double mutant, indicating that chromosome axes are required for DSB formation and DSB repair, respectively. Taken together, these results suggest that AFD1 serves as a basic foundation of chromosome axis that facilitate the recruitment of DSY2 and ASY1, and the chromosome axes formed by these proteins are required for recruitment of DSB formation factors.

P144

Analysis of ten meiotic mutants of maize

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Maize is an excellent model to study meiosis thanks to excellent cytology and a large collection of meiotic mutants (Cande et al., 2009). We are analyzing five mutants affected in meiotic prophase I and recombination (spo11-1 (cloned), as1, dsy9902, dsy9905, and meiN2415) and five mutants that have defects in the condensation of meiotic chromosomes (afd1 (cloned), elongate1, ms43, sticky and Mei025). Our detailed analyzes of molecular cytogenetics will allow to concentrate on several aspects of the early meiotic recombination process and the chromosomal condensation that occurs during the progression of prophase I of meiosis. We are also investigating how meiotic recombination coordinates with the progression of chromosome conformation by studying an essential part of the collection of the maize meiotic mutants. This project allowed to save (re-propagate) ancient seeds of eight of these mutants (except Mei025 and sticky). We are mapping and trying to clone the corresponding eight genes. We are also analyzing them with new cytogenetic and proteomic tools. An applied aspect of this research in progress is to valorate this collection of mutants with the aim of introducing a clonal reproduction in maize. The possibility of manipulation of maize sexuality to maintain heterosis (vigor of the hybrids) was revived by the development of transgenic lines of Arabidopsis capable of clonal reproduction similar to apomixis (Marimuthu et al., 2011) (Ronceret and Vielle-Calzada 2015) and the recent clonation of the haploid inducer stock6 (Kelliher et al. 2017, Gilles et al 2017, Liu et al. 2017). We have initiated crosses between non-transgenic meiotic mutants to produce an 'apomeiotic' maize, the first step for the generation of maize seeds with a clonal reproduction that can maintain heterosis.

Funding acknowledgement: UNAM-PAPIIT

P145

Dynamics of meiotic spindle assembly in *Zea mays*

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The success of an organism is contingent upon its ability to faithfully pass on its genetic material. Chromosomes must be correctly segregated between dividing cells, a process that is particularly critical in the meiotic divisions that generate an organism's gametes. The machinery used to pull chromosomes apart is the spindle, a bipolar, microtubule-based structure. Male maize meiocytes lack many of the features that govern the assembly, organization and positioning of the spindle, so we investigated the dynamics of the spindle assembly process in wild-type meiotic cells. Using fluorescently-tagged lines, spindle assembly was observed in meiosis I and II via live microscopy. We found that meiotic spindle assembly is characterized by the following steps: collapse of the nuclear envelope with associated microtubules, a re-organization of these microtubules into a bipolar shape, lengthening of the spindle and focusing of the poles. Cells frequently formed tripolar or multipolar spindles; approximately half of all assembly events initially formed a tripolar spindle. Tripolar spindles were re-organized into correctly shaped bipolar spindles during prometaphase chromosome congression. Spindle also frequently failed to fully focus their poles before transitioning from metaphase to anaphase. The frequency of substantial errors in assembly and their subsequent correction before anaphase suggests an active and essential role for the spindle assembly checkpoint in the progression of meiosis. However, the initiation of chromosome segregation in the presence of unfocused poles suggests a leniency of the checkpoint that allows progression despite minor errors.

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P146

Frequency of abnormal chromosome 10 in tropical landraces of *Zea mays*

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Abnormal chromosome 10 (Ab10) is a selfish maize chromosome that promotes its own inheritance to future progeny over normal chromosomes. Extensive genetic analysis of this abnormal meiotic drive system has been carried out mainly on agricultural and research landraces of North America and Mexico. The presence of Ab10, however, has not been widely studied in tropical maize landraces. We investigated the frequency of abnormal chromosome 10 in a variety of *Zea mays* tropical landraces from five South American countries. Only two of 43 tropical landraces tested positive for the presence of Ab10. Of the two positive landraces, 67% of Peruvian individuals, and 60% of Venezuelan individuals contained Ab10. Currently, three types of Ab10 exist and are determined by their cytological structure. Fluorescence *in-situ* hybridization (FISH) revealed that the Peruvian population carries Ab10 variant type-III, which is the most prevalent type. In addition, geographic observations revealed that both Ab10 positive landraces were cultivated from regions at average altitudes of 120m and 115m. These findings support previous research reporting high occurrences of abnormal chromosome 10 within populations found at lower altitudes.

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P147

Meiotic crossovers in maize: the interfering and non-interfering recombination pathways have different landscapes

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The formation of crossovers in meiosis ensures a proper segregation of homologous chromosomes, but it also shuffles the allelic combinations, generating in each product a mosaic of the homologous pairs. Thus crossovers are the physical cause of recombination while providing the variation upon which natural and artificial selection operate. In maize, the majority of crossovers come from the interfering pathway, while the remaining 15% or so of the crossovers come from the non-interfering pathway (Falque et al., Plant Cell 2009). Direct observations in tomato have revealed that these two pathways have different recombination landscapes, with crossovers in the peri-centromeric regions coming mainly from the non-interfering pathway (Anderson et al., PNAS 2014). Unfortunately similar experiments to identify the pathway via which each observed crossover was formed have not been performed in any other plant species. Hence to understand the properties of the two recombination landscapes in maize, we resort to data analysis and modeling.
Our first approach relies on a test within a novel statistical framework. By performing a model-independent analysis of maize data (Anderson et al., Genetics 2003), we are able to reject the hypothesis that crossover interference operates homogeneously in genetic coordinate space and thus points to having different shapes of landscapes for each pathway, as is known to be the case in tomato. Our second approach, based on modeling, allows the two pathways to have different recombination landscapes and takes the crossovers of the first to be generated by the so-called Gamma model. By fitting the crossover data (not knowing whether a crossover was formed as a result of the interfering or non-interfering pathway), we infer the two separate landscapes in maize for each of the 10 chromosomes. The trends arising in these predictions are then compared with what is known in tomato.

P148

Visualizing plastid sequences present on the B Chromosome of maize

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In maize, B chromosomes are extra chromosomes that exist in association with the normal chromosomal set and are not essential for the survival of the organism. Through mitotic drive, a two-part accumulation mechanism during pollen maturation, B chromosomes are maintained in populations and can be present in variable numbers. The exact origin of the B chromosome is uncertain; however, previous research has determined its structure to be mostly heterochromatic, containing a collection of repetitive sequences from nuclear and organellar genomes. Using fluorescent *in situ* hybridization (FISH), we have shown the presence of mitochondrial DNA on B chromosomes. Sequence analysis suggests that plastid DNA sequences are also present on B chromosomes. This research focuses on using FISH analysis to visualize plastid sequences present on the B chromosome of maize.

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P149

Doing genetics research in the classroom: CRISPR-Cas9 and cold stress in plants

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Integration of authentic research experiences is crucial to providing all students with equal access to research opportunities. To engage students in plant genetics research, we developed a laboratory series guiding students through the process of creating CRISPR-Cas9 vectors and producing mutant Arabidopsis plants. Despite being a relatively new technology, CRISPR (Clustered regularly-interspaced short palindromic repeats)/Cas9 has proven to be a revolutionary, efficient, and precise mechanism for editing genomes through targeted mutagenesis and it could be used as an effective draw for students' interest in genetic and biotechnology. In our system, the students can choose the genes that they would like to knock out or the target genes could be drawn from research projects that involve testing the effects of mutations in candidate genes. We developed all learning materials using the protocols from Voytas Lab (Čermák et al., 2017) and tested them on Arabidopsis genes homologous to maize genes involved in cold stress response, as well as several Arabidopsis genes with well characterized phenotypes as controls. The vectors produced by students were analyzed by restriction digests, colony PCR, and sequencing, demonstrating the success of vector assembly. The T0 plants were transformed with T-DNA transformation vectors for all five selected genes and T1 transformed seeds were selected. The results of the phenotypic screens show the success of CRISPR-Cas9 protocols we implemented and the suitability of this approach to a regular laboratory course. The results of student learning assessment indicate the effectiveness of our approach.

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P150

Evaluation of genetic progress in grain and silage corn afforded by the renewal of varieties in France over the past 30 years

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Genetic improvement made on different grain corn traits over the past 50 years in France has been the subject of several studies based on specific trials comparing varieties, which have marked the history of maize since the 1950s (Derieux *et al.*, 1987; Welcker *et al.*, 2011). Annual yield gain results align with the estimations obtained in the United States (Duvick *et al.*, 2005). Therefore, regular updating of the references for each earliness group in grain corn and corn harvested as silage (Baldy *et al.*, 2017) is important to (i) evaluate selection dynamism in each earliness group, (ii) show farmers the effect of genetic progress in the evolution of agricultural yields in a context where the increase in national average yield has slowed since the 2000s, (iii) determine the expected levels of progress for different variety selection criteria, particularly in the context of discussions on variety registration rules. The results database of the last 30 years from the Arvalis – Institut du Végétal maize variety evaluation network (10 to 35 validated trials/year according to the variety earliness group) represents valuable material for proposing, with the help of statistical fitting for incomplete data series, an assessment of annual genetic progress in yield, earliness and lodging. This poster describes the methodology and assessment of genetic improvement of the varieties offered annually to farmers in France.

P151

Fifty years of mirrored in situ and ex situ conservation of Mexican maize landraces: socioeconomic dynamics and genomic diversity

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Although *in situ* and *ex situ* strategies for genetic resource conservation are considered complementary, the evidence available on the topic and specifically on maize is indirect. Our objective is to make a straightforward comparison of how the diversity of Mexican maize landraces has been conserved in genebanks and in farmers' fields over the last 50 years. To do so, we focus on a set of 93 accessions of maize traditional varieties collected in 1967 from 66 families from the state of Morelos, Mexico and stored at the CIMMYT Maize Germplasm Bank. Passport information from each accession included its common name, pictures of five representative ears, village where the sample was collected, name of the farmer who donated the sample and number of collected ears. With this information we traced back the same farmer families 50 years later, in 2017, and collected new samples from the accessions that had been conserved *in situ*. Thirteen families had conserved 14 accessions under constant cultivation during the 50-year period. We collected 11 of these accessions and assembled 11 *ex situ* and *in situ* accession pairs for genetic characterization and comparison. DNA from these populations was genotyped using double restriction site associated DNA markers (ddRAD). We opted for a mixed approach in which both single plants (10 individuals per accession) and pooled samples (2-3 pools of 30 individuals per accession) were compared. Through in depth interviews we also documented the history of loss or conservation of these maize traditional varieties among the families who donated the samples in 1967. By bringing genetic and socioeconomic approaches together our aim is to assemble a comprehensive picture of how variation in these maize populations has evolved over time and to shed light on which could be the most appropriate strategies for their conservation.

Funding acknowledgement: Sant'Anna School of Advanced Studies, Department of Social Sciences Wageningen University

P152

Learning how to rescue a landrace: A study of the giant maize, Jala, and the community who grows it

(submitted by Denise Costich <d.costich@cgiar.org>)

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Maize landraces are famous for their high degree of local adaptation and specialization, resulting from many cycles of selection by the communities who grow them. One of the most famous examples is the giant maize, "Jala," named after the valley where it grows in the central western state of Nayarit in Mexico. Even though this variety has distinctive agronomic and culinary attributes that are expressed and valued mainly at the local level, there has been a reduction of the total metapopulation size, from millions of plants grown in large, contiguous land holdings in the 1950s, to relatively few (<100) small, isolated individual smallholder farmer plots since the 1980s. In 2017, we carried out a field trial near the village of Jala, evaluating Jala landrace collections from four local farmers versus 14 historical materials conserved in the CIMMYT Germplasm Bank. In addition, we conducted a socioeconomic survey to understand Jala maize dynamics, replicating a survey carried out in 2001 by Rice (2004; 2007). This multi-disciplinary approach is helping us to identify the main biological (genetic and agronomic) and socioeconomic factors that affect Jala maize conservation. The field trial clearly identified which of the historical collections could provide useful germplasm for a future breeding program. Socioeconomic results indicate that although Jala maize is multi-purpose, displaying a variety of culinary uses, as well as providing quality forage for livestock, the main reason the farmers keep conserving it is tradition. This tradition has been reinforced by an annual local contest that rewards the producers of the largest ears of Jala maize. However, these first findings also indicate that a more holistic and aggressive strategy of conservation is needed to truly rescue the Jala maize landrace from the current trend toward its eventual disappearance from the Jala Valley.

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P153

The maize collection of CIMMYT's germplasm bank: Promoting the conservation, use and study of diversity

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This is an exciting time for the Maize and Wheat Germplasm Bank at CIMMYT. DNA sequence data are now available for diversity analyses of the collections, from our colleagues in the Seeds of Discovery Project. The implementation of the GRIN Global database management system is nearing completion. Our germplasm is being used for gene discovery projects for emerging diseases, such as Maize Lethal Necrosis (MLN) and Tarspot Complex. The CIMMYT Maize Lines (CML) collection has never been better documented, both phenotypically and genetically. Innovations in seed processing and regeneration that focus on enhancing the maintenance of genetic diversity and maximizing efficiency, are currently being tested. We are building a new screenhouse dedicated to the regeneration of wild relatives. Strengthening our relationship with the USDA Maize Collection in Ames, Iowa has accelerated many of these improvements. We continue to participate in research on community-centered landrace conservation and the development of community seedbank networks, in partnership with the MasAgro Program. In order to provide the maize genetics community with a more detailed understanding of the germplasm we hold in trust for humanity, a diversity tree analysis of the collection will be presented. Another activity underway to promote use by breeders and researchers includes the identification of subsets with special types of associated data, such as kernel characteristics or disease resistances. This is part of an effort throughout all CGIAR genebanks to connect decades of characterization data with the seed accessions, to promote gene discovery and related genetic research. We acknowledge the strong support from the Global Crop Diversity Trust, as well as its unwavering leadership in promoting and giving direction to our mission through the CGIAR Genebank Platform.

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P154

NSF Plant Transformation Workshop

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Plant transformation has been a bottleneck in advancing plant functional genomics study and genome editing. Transformation of recalcitrant cereal crop species has been challenging. The National Science Foundation - Plant Genome Research Program sponsors this training workshop. The goal of this workshop is to provide attendees with hands-on experience in *Agrobacterium*-mediated transformation of cereal crop species. Attendees will have the opportunity to walkthrough advanced protocols for transformation of cereal crops with focus on three recalcitrant cereal species; including *Zea mays* inbred lines, *Sorghum bicolor* public genotype, and *Brachypodium distachyon* public genotype. Trainees will have the opportunity to learn how to utilize plant morphorgenic regulator genes to transform B73 as well as the best practice for cereal transformation. In addition, the workshop will offer two lectures and host a discussion forum. The first lecture will focus on the mechanism of plant somatic embryogenesis whereas the second lecture will center on how to establish and implement cereal transformation systems. The Plant Transformation Core Facility at University of Missouri, Columbia, MO, USA, will host this workshop from July 30 to August 3, 2018. For workshop pre-registration (free), please visit Plant Transformation Core Facility website at https://plantsciences.missouri.edu/muptcf/workshop and for any workshop update. Seats are limited. Pre-registration is required by June 20, 2018 to secure your spot and facilitate our workshop organization. Please contact Dr. Zhanyuan J. Zhang (zhangzh@missouri.edu) for any workshop related questions.

Funding acknowledgement: National Science Foundation (NSF)

P155

Maize annotation jamboree on last B73 RefGen_V4 assembly

(submitted by Cristina Marco <marco@cshl.edu>)

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The first genomic Annotation Jamboree for the current reference Maize B73 (B73, RefGen_V4) was held on December 4-5, 2017 at Cold Spring Harbor Laboratory (CSHL). Sponsored by the NSF-funded MaizeCODE (IOS-1445025) and Gramene (IOS-1127112) projects, the Jamboree developed a model for how graduate students can be involved in improvement of Maize gene models. This event was a proof-of-concept for similar future efforts for improving annotations in Maize inbreeds, sorghum, and other important crops, as well as for inclusion of undergraduates. This event brought together participants from seven US and one International institution (University of Tokyo). 10 graduate students and one postdoctoral fellow participated in this two-day event.

Students grouped in pairs checked the accuracy of five Maize gene families: PIN, GH3, ABC, TCP and ORC. From these gene families, 'suspicious' MAKER-P-generated annotations were identified based on their annotation edit distance (AED) and quality indexes (QI2). Working independently on the same set of models, students found that 19% of the genes they looked at needed manual annotation including setting exon boundaries, identification of non-canonical splice sites, missing exons or missing UTRs. From the genes that were tagged with the AED and QI2 parameters, the primary gene model was corrected in approximately 70% of them and the other 30% represented multiple isoforms which were difficult to curate. Further tests are being performed to determine if this method might be used to better predict gene models for manual curation and develop workflows to automatize the process.

We describe conclusions, approaches, and identified improvements that will ultimately be fed back to MaizeGDB curators as updates.

Funding acknowledgement: National Science Foundation (NSF)

P156

A candidate gene approach to identify markers associated to Fusarium Kernel Rot resistance in maize

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Fusarium verticillioides is a major maize pathogen and it is associated with various symptoms of Fusarium ear rot on maize. Resistance during seed germination is part of the plant-pathogen interaction but this aspect has been poorly investigated. The best way to contrast this disease is the development of immune maize genotypes. Several genes are reported as important for disease resistance, but their role against *F. verticillioides* is still not understood. Point mutations, small insertions and deletions are responsible for the evolution of resistance genes within a species. In this work, the presence of sequence polymorphisms in maize *R* genes, encoding NBS-LRR proteins, and in *Fusarium* induced genes, identified by a RNAsequencing experiment, were analyzed in resistant and susceptible lines. Then, the detected polymorphisms were used to genotype a panel of 267 lines with phenotypic data for *F. verticillioides* resistance obtained using a rolled towel assay.
For *R* genes, 171 SNPs and 11 INDEL and for RNAseq-derived genes 104 SNPs and 11 INDEL were found in 38 and 17 genes, respectively. Forty polymorphisms were selected to genotype the panel of 267 maize inbred lines. Genotypes were correlated to phenotypic data and four markers were associated to five different phenotypic traits. All markers significantly associated with phenotypes were present in genes with a clear function in disease resistance. These findings will contribute to understanding the maize-*F. verticillioides* pathosystem interaction.

Funding acknowledgement: Doctoral School on the Agro-Food System (Agrisystem) of Università Cattolica del Sacro Cuore (Italy), European Union's Horizon 2020 Grant Agreement No. 678781 (MycoKey)

P157

A Novel Mating Design Provides High Power to Detect Epistasis in Maize

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Quantitative phenotypes are determined by a myriad of genes functioning in concert within an organism. The phenomenon of two or more of these genes interacting to influence a phenotype, known as epistasis, is critical for understanding genetic architecture. However, the complexity of epistasis makes it hard to detect. From a computational standpoint, searching all pairwise interactions in a dense marker panel is a daunting task. From a statistical standpoint, appropriately accounting for multiple-testing corrections can make this nearly impossible. Here, we describe a crossing scheme that can be used to develop Epistasis Mapping Populations (EMPs), which leverage search space reduction to powerfully detect epistasis. Our strategy employs near isogenic lines (NILs) crossed in a simplified half-diallel mating scheme and backcrossed to the recurrent parent. Ultimately, a collection of triplets can be formed that allows the straightforward evaluation of whether or not introgressions in the founding NILs interact epistatically. The efficacy of this approach to detect epistasis was evaluated using both simulations and a field trial. We simulated an EMP based on B73 and Mo17 and compared its power to detect epistasis to that observed in the commonly-studied Intermated B73 x Mo17 (IBM) population. Results from field trialing in the summer of 2017 using two replications in two locations suggest that epistasis may be widespread for several maize traits, including days to anthesis, days to silking, plant height, ear height, and ear number, but barely impact additional traits including node number. Our analysis using simulated and real maize EMP populations demonstrates that this approach can be powerful for detecting epistasis in maize.

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

P158

A RNA-Seq atlas on the founder lines of the MAGIC population "BALANCE", a supplementary tool for gene discovery

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LD mapping in the BALANCE panel (derived from the BALANCE population) offers a sufficient resolution to reduce traits associated genomic regions to several putative causal genes. Access to expression data is an additional step to understand which gene(s) could explain phenotypic variations. In our strategy to aggregate complementary layers of information around our BALANCE panel, a significant sampling experiment was conducted in greenhouse on the founder lines in order to answer several questions: Are genes differentially expressed among founder lines, tissues or developmental stages? Is gene expression affected by an abiotic stress? Can we detect splicing variants or PAV? Eight hundred plants from testcross progenies were grown into 2 water regimes. More than 1000 samples were collected at 6 developmental stages in 3 different tissues (leaf, silk, kernels) under well-watered and water deficit situation. To date, transcripts of 93 leaves samples, collected at 0/5/15 days after anthesis in optimal and stress conditions, were sequenced and analyzed. After raw data processing (read normalization and alignment on B73 V2 sequence), 23 749 genes were considered as expressed among which 5 253 were differentially expressed. Clustering of leaf samples results shows an obvious separation between well-watered and water deficit samples. A weighted gene co-expression network analysis (WGCNA) was used to identify highly co-expressed genes involved in drought-stress responses. Gene-ontology enrichment, differentially expressed genes mapping and cross-network comparison (with Arabidopsis and Maize co-expression networks from literature) were then performed to select a stress related module of genes. A list of 351 candidate genes with high connectivity parameter (hub genes) were selected for further evaluation, especially by LD mapping.

P159

Agronomic trials characterization and clustering using water and nitrogen stress indices simulated with a crop model

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Evaluation of cultivars performances, either during the breeding process or the post-registration phase, are largely based on multi-environment trials (MET) consisting in testing genetic material over a broad range of genotype x management x environment (location and year). Analysis of these data allows estimating the average performances of cultivars or lines across different agroclimatic scenarios. These METs are also commonly used in plant genetics research to identify quantitative trait loci (QTL) involved in plant performances. A significant part of the phenotypic variation explained by those QTL comes from QTL×E interactions which often involve different responses of genotypes to biotic or abiotic stresses. Despite some of the environmental factors can be controlled in a field trial with proper crop management options (sowing date, fertilization, irrigation), a large number of other factors are out of control (climatic conditions) and the chosen management options may sometimes fail. Therefore, it is often difficult to determine what really happened in terms of the dynamics of the limiting factors that occurred in a trial. Here, we present the a posteriori characterization of the MET carried out on a dent panel in the Amaizing project. This panel was tested over eight locations in France during two years and under different crop managements (optimal condition, low nitrogen, early sowing, rainfed). We used the CHN crop model to calculate high temperature, nitrogen and water stress indicators based on on-site meteorological records, soil characteristics and crop management information. This led to revisit trial classification due to unexpected water or nitrogen stress. Trials were grouped based on the dynamics of these stresses. QTL×E interactions within groups are expected to be lower. This may improve our understanding of the QTL×E interactions and finally provide knowledge to breeders on specific adaptation QTL related to high temperature, nitrogen and water stress tolerance.

Funding acknowledgement: Agence Nationale de la Recherche (ANR)

P160

AMAIZING, a project on Maize Integrative Genomics supported by the French program "Investments for the Future"

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Maize is cultivated annually on 3 Mha in France (23 Mha in Europe). In addition to grain and silage for animal feeding, France produces hybrid seed on 58 000 ha. The AMAIZING project (https://amaizing.fr/en/) was started in 2011 with the support of the French National Research Agency (ANR) and will end in 2020. In view of breeding next generations of varieties, it aims at increasing knowledge on maize (epi)genomic diversity, genetics of adaptation to abiotic constraints (cold, drought, limited nitrogen supply) and heterosis, and to develop genetic and (eco)physiological modelling.

The project groups 24 partners with complementary expertise: 15 public research laboratories at universities or national research organizations, 7 seed companies, a biotechnology company, a technical institute and seed certification organism. It is organized in 9 workpackages, led by the authors of this poster. The experimental workpackages target (posters at MGC 2018 indicated):

- Characterization of maize genomic and epigenomic variation, its effect on gene expression and contribution to European maize adaptation (WP3; P7, P29, P147, P254)
- Optimized genetic resources, genotyping and statistical approaches for GWAS mapping and genomic selection (WP4; P176, P177, P185, P194, P197, P199)
- Genome-wide analysis of environmental adaptation (WP5), based on hybrid diversity panels evaluation for proteomics (P191), metabolomics, platform phenotyping (P100, P103, P239), multi-location field trials characterized for environmental data (P159),
- Functional validation and fine characterization of main loci detected for yield and adaptive traits (WP6, P239)
- Modelling and integrative approaches: environmental adaptation, ecophysiological modelling in view of genetic gain analysis and prediction (WP7; P79, P100, P103)
- Application in breeding programs and variety evaluation (WP8).

These scientific workpackages are supported by transversal workpackages dedicated to management (WP1), bioinformatics (WP2, see P232) and communication (WP9). Information on project structure and results can be found at https://amaizing.fr/en/

Funding acknowledgement: INRA, ANR-10- BTBR-01 (Amaizing), France Agrimer

P161

Analyses of genetic variation associated to deep planting resistance in maize revealed genes controlling development, growth, and adaptation to soil conditions

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Crop adaptation to drought and high temperature depends not only on genetic and phenetic traits and their interaction with the physical, chemical, and biological environments, but also to humans through their interaction with agricultural management practices. Few studies have focused in understanding the extent at which selection by agricultural management modifies the phenotype and genotype of crop populations and much less how all of these leads to stress adaptation. To better understand these interactions we studied deep planting resistance in inbred maize lines, hybrids, and native landraces of Mexico. Deep planting is used in semiarid regions of the Mixteca Alta, Trans-Mexican Volcanic Belt, and the US southwest to take advantage of residual soil humidity. Analyses of a large panel of Mexican landraces uncovered two developmental patterns that accounted for deep planting resistance: long mesocotyls without plumular growth or short mesocotyls with rupture of the coleoptile by the plumule. We found a strong correlation between growth and developmental patterns with planting depth (deep versus shallow) practiced by the donating farmer, supporting the idea that deep planting selected in favor of long mesocotyls and against rupture of the coleoptile by the plumule prior to seedling emergence. GWAS of mesocotyl length variation under deep planting revealed a variety of transcription factors and small molecule transporters associated to photomorphogenesis and adaptation to soil conditions below 20 cm depth. GWAS of plumular growth variation under deep planting identified transcription factors involved in morphogenesis, cell differentiation, and regulation of growth, in addition to kinase receptors, metabolic enzymes, and transporters. Some of these markers were validated by QTL, resequencing, and transcriptome analyses. These molecular markers will be fundamental to understand the evolution of traits relevant to climate change adaptation that were selected during domestication and/or improvement by traditional and sustainable agricultural methods

Funding acknowledgement: CONACYT-Mexico, PAPIIT-UNAM-Mexico

P162

BALANCE, a powerful MAGIC population for the identification of genetic determinants involved in the variation of traits of interest in maize

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LD mapping has become a method of choice to identify genomic regions involved in the variation of traits of interest in various species. To overcome the problem of genetic structure in association panel, BIOGEMMA has developed a MAGIC population from 16 historical lines representative of the genetic diversity used for hybrid production in temperate regions. This MAGIC population was used to produce a panel of ~400 DH lines: the BALANCE panel. For LD mapping, this panel has the advantages of a typical diversity panel (large genetic diversity, rather low LD) without the main handicaps (strong genetic structure and heterogeneity of parentage relatedness). Simulations of phenotypes showed that the panel had enough power to detect QTLs explaining 5% of the phenotypic variation of a quantitative traits; that is 2 times higher than that in a panel of elite lines and slightly higher than that in a maize diversity panel. To reap all the benefits of this panel for gene discovery, BIOGEMMA capitalizes on many "omics" aspects, aggregating complementary layers of information in an integrative strategy. For several years, the BALANCE panel has been extensively phenotyped for agronomic traits under water deficit (more than 20 field trials). The panel is also being phenotyped with innovative tools (UAV, root phenotyping platforms) for physiological traits. Moreover, the 16 founder lines were sequenced and several millions of SNPs were identified and imputed on the entire panel. Finally, a transcriptomic experiment was conducted on the founder lines (more than 1000 samples) to identify genes differentially expressed under well-watered and water deficit situation. This experiment provided candidate genes and enabled the development of a gene atlas. Phenotypical, transcriptomic and genomic aspects are detailed in three different posters.

P163

Changes in carotenoid and tocopherol content in maize grain during cold storage

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Carotenoids are yellow, orange, or red plant pigments, the second most abundant pigment in nature. They are found in most plant organs and tissues, although are not visible in green tissues, due to presence of chlorophylls. In dormant grains, an non-photosynthetic tissue, carotenoids are located in plastids, improving integrity of membranes, preventing degradation of nutrients, and protecting against free radicals during seed aging. They comprehend two chemicaly similar groups of carotene and xanthophyllus. Lutein and zeaxantin are the most abundant carotenoids in maize, followed by smaller amounts of ß-carotene. The aim of present work is comparison of five maize landraces differing in carotenoids and tocopherols content in grain, after cold storage in gene bank, for 5, and 30 years, as well as in samples after their field regeneration. ß-carotene content in the oldest samples decreased in range of 81.57-91.86% compared to newly regenerated samples, while lutein+zeaxantin content reduction was in range of 66.66-83.28%. Considering tocopherol content, the greatest lowering was evident for α-tocopherol (52.9%), folowed by 37.23% decrease in ß+γ content. The smallest changes were in δ-tocopherol content of the oldest seeds, but five-years old samples had smaller content in newelly regenerated seeds. The greatest decrease of about 50% was evident after five years of cold storage in carotenoid content, followed by slowlier reduction with seed ageing. The similar trend was observed for tocopherol content. Carotenoids of heterotrophic organs and seeds are with larger diversity compared to those involved in photosynthesis, and are less investigated. Their protective function and decelerating againg, together with increased importance in biofortification breeding programs, gave higher importance to maize landraces stored in genebank, towards achivement of target nutritional content in maize grain.

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P164

Co-regulation of ZCN8 and ZCN12 underlies Maize flowering variability

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When to flower is a critical decision affecting maize productivity for its connection to the different local cropping environments. Still very little is known about the mechanisms regulating flowering time in maize and how their genetic variability accounts for the different phenology observed. The florigen genes, encoding a systemic flowering signal, are critical promoters of the floral transition. We will present expression and functional data supporting the contribution of two maize florigen genes - ZCN8 and 12 - in the maize flowering time diversity. From a panel of 320 temperate maize lines grown in a phenotyping greenhouse we could produce quantitative, tissue-specific and temporal information of different florigen genes expression which could be related to the flowering phenology. Our results demonstrate a robust negative correlation between ZCN8 transcript levels and time to flowering. Moreover, variability in ZCN8 expression can be confidently related to flowering time under field conditions. ZCN8 eQTLs regions overlap with flowering time QTLs detected under both greenhouse and field conditions, suggesting that differences in ZCN8 levels contribute to modulate flowering.
Our dataset offers opportunities to evaluate the role of other ZCN8-like genes to flowering. Differences in the accumulation of ZCN12 are determined by a major effect eQTL, mapping in the first exon of ZCN12 itself. Molecular markers allowed us to distinguish two ZCN12 allelic variants, one of which transcriptionally active, and whose accumulation is negatively correlated with flowering time. Strikingly, expression of this allele version across our lines closely follows that of ZCN8, indicating a common regulatory mechanism. Modelling studies support the existence of a two tiers florigen system in maize, whereby accumulation of ZCN8 promotes the activation of ZCN12. Our data suggest that different combinations of ZCN8 and ZCN12 expression underpin developmental variability which could enable further flexibility of maize cultivation to different environments.

Gene / Gene Models described: *ZCN8 ZCN12*; GRMZM2G179264 GRMZM2G103666
Funding acknowledgement: Ceres initiative of Fondazione Cariplo and Agropolis Fondation

P165

Computer-vision into the biology of fungal-leaf interactions in maize

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Quantitative disease resistance (QDR) is a sustainable solution for disease management in crop production systems, but the biology of QDR is only beginning to be understood. Using whole plant, field-scale studies and multiple genetic methods, our team has identified genes and elucidated the molecular genetic basis of QDR to fungal pathogens of maize, including *Cochliobolus heterostrophus* and *Setosphaeria turcica*. Highlights of these genetic studies will be presented. To understand QDR at the tissue-level, a semi-automated 3D macroscopic microscopy platform was developed and used to microscopically image multi-millimeter areas of pathogen infected leaf tissue. Using this image data, the U-Net convolutional neural network was adapted to computationally segment cells of the host leaf and mycelial networks of the pathogen. This process generates complex, cell-level 3D morphology data, which we are currently analyzing (and figuring out suitable methods for analysis). Specifically, we are extracting high level plant-pathogen information such as features of the neighborhood of host cells and stomata surrounding fungal penetration events, as well as fungal infection network sizes and shapes. Macroscopic microscopy was also complemented with higher-resolution images captured by multi-photon confocal microscopy in order to distinguish specific aspects of fungal-leaf interactions. We find that infections by *Cochliobolus heterostrophus* have a similar depth of penetration into the leaf tissue of maize lines with contrasting levels of resistance, but the infection networks are differentiated by their spread within the subepidermal space. The two pathogens show very different infection strategies that produce macroscopically visible lesions which are either congruent or incongruent with the localization of the pathogen. Together, our work is providing unique insight into fungal pathogenesis on maize while contributing to the advancement of cellular phenomics in plants. The latest results from this work will be presented.

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P166

Construction of maize grain moisture content related near-isogenic lines for developing SNP molecular marker

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Maize is an important food crop, and is also used as an ideal material for plant genetics and functional genome research. With the completion of the maize genome sequence, studies on maize functional genomics become more and more widely. The near-isogenic lines (NILs) are the important materials for the construction of molecular genetic maps, QTL location and molecular markers-assisted breeding. Maize species Zhengdan 958 (Zheng 58 x Chang 7-2) and Xianyu 335 (PH6WC x PH4CV) are widely cultivated in China, showing differences on growth period, plant architecture, grain filling rate, grain dehydration rate, etc. Here, we create Zheng 58 NILs and Chang 7-2 NILs by introducing PH6WC and PH4CV background. These NILs were obtained by consecutive selective backcrosses and then selfing. There were highly significant differences in growth period, flowering date, plant height, grain filling rate, grain dehydration rate, etc among these 600 NILs. Genotypes of these NILs were identified by using maize 55 K SNP array. And genome wide association study (GWAS) was used to detect genetic factors for governing grain moisture content (GMC), grain filling rate (GFR) and grain drying rate (GDR). And SNP molecular markers will be developed for molecular marker-assisted selection breeding.

P167

Creation of subgroup specific haplotype blocks and libraries

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Over the years defining haplotype blocks has been shown to be a very useful tool in genomic approaches. Fields of application range from the detection for regions under positive selection to all sorts of statistical application that make use of variable reduction. In contrast to traditional methods we do not use a population-wide measure (linkage disequilibrium, LD) but instead screen subgroups of haplotypes for common variants and thereby focus on linkage. We define a haplotype block as a sequence of alleles and only those haplotypes with a similar sequence are considered to be part of a block. Because of this, blocks can overlap and not every position has to be part of a block. Out of these haplotype blocks we construct a haplotype library representing a large proportion of genetic variability with a limited number of blocks. Depending on the application, different optimization goals of the haplotype library (e.g., the identification of shared segments between different breeds) are possible. Our methods are implemented in the so far unpublished R-package HaploBlocker. By applying this method we reduce a dataset comprising of 313 DH-lines in a European Landrace (75k SNPs, chromosome 1) to 370 haplotype blocks with an average length of 2'015 SNPs that represent 93.7% of the dataset. In contrast blocks derived via HaploView have an average length of 89 SNPs – this difference becomes even more severe when comparing the block length of a dataset with two landraces (1'705 SNPs vs. 24 SNPs) since LD is heavily reduced in more diverse panels whereas linkage can still be detected similarly. By using haplotype blocks instead of SNPs, local epistasis interactions can be modelled naturally and the typical p>>n-problem in genetic datasets can be reduced, which enables the application of a wide variety of new methods for further analysis.

Funding acknowledgement: Federal Ministry of Education and Research (BMBF; project: MAZE; http://www.europeanmaize.net/)

P168

Deployment of a novel phenotypic platform based on biomechanical engineering principles provides novel insights into stalk lodging in maize

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Stalk lodging in maize poses an important production challenge worldwide. While these losses are preventable through genetic improvement of stalk lodging resistance (SLR), such efforts have been impeded by the lack of a robust phenotyping method. Traditionally used testing methods like lodging incidence count and rind penetrance resistance are prone to inconsistent testing parameters, heavy environmental influence, and one-dimensional analysis. Many intermediate phenotypes contribute to SLR that are not accounted for in the currently available phenotyping methods. We have employed a novel phenotyping platform, developed with extensive insight from structural engineering that evaluates the ability of an individual maize stalk to bend before failure (bending strength). Additionally, we identified key intermediate traits putatively underlying SLR and recorded these traits to obtain insights into their relative impact on SLR. Testing materials included a diversity panel, divergently selected high and low rind penetrance resistance (RPR) populations, and hybrids from the G2F initiative, collectively spanning over 500 genotypes. Deployment of the new platform was successful in providing a variety of both accurate and consistent measurements for a multitude of intermediate traits related to SLR. This data revealed interesting relationships among the intermediate traits and provided novel insights into the structural and anatomical features contributing to SLR. Importantly, we show that simultaneous measurements of multiple structural features by the new platform provide a comprehensive picture of SLR compared to traditional methods like RPR. GWAS analysis on the diversity panel identified serval novel associations for bending strength and the intermediate traits. Importantly, many of the associations were shared between multiple traits demonstrating shared genetic regulation of these traits. We demonstrate that the platform is very effective in providing a reliable estimation of SLR both for phenotypic selection and for generating mechanistic insights into the genetic regulation of SLR in maize.

Funding acknowledgement: United States Department of Agriculture (USDA)

P169

Design Thinking and Data Mining in Plant Breeding

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Plant breeding is enhanced by integrating different scientific innovations and enabling tools. One major challenge that comes with the wide adoption of genomics and biotechnologies is to rethink and redesign the breeding programs at different stages and different scales. The essence of this new wave of breeding methodology research is to effectively identify and exploit genotype to phenotype relationship so that desirable cultivars are continuously and efficiently developed. Design thinking is a human-centered mindset and problem-solving methodology that is being widely adopted to address complex problems. Data mining, successful in many other areas, provides technical solutions to address this question, particularly when findings are integrated into the designing process. Enhanced by design thinking and data mining, genomics-assisted prediction may reshape the plant breeding pipeline by enabling the efficient exploration of the enormous inference space of genetic combinations, environment combinations, and performance dynamics. We propose three essential components to streamline the breeding in the post-genomic era: better product creation (BPC), knowledge discovery from data (KDD), and optimal program design (OPD).

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

P170

Development of a new high throughput 105K presence/absence variation genotyping array for quantitative genetic studies

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Structural variation are pervasive in plants. In the last decades, thousands of copy number variations (CNV) have been discovered among the maize genome, notably Presence/absence variation (PAV) of some genomic sequences.
We developed a new genotyping array to genotype PAV in a large set of individuals in order to explore their contribution to genetic diversity and trait variation in maize.
First we discovered 120,178 PAV from the resequencing data of 3 inbred lines (F2, C103, PH207) compared to the B73 reference genome. Second, we designed 26 million probes in order to be able to genotype PAV breakpoints as well as PAV internal sequence. Among them 662,772 were selected and used to design an Affymetrix Axiom array. These probes make it possible to genotype 105,927 PAV sequences from 35bp to 127kbp long. Among these PAV, 25% are deletions and 75% are insertions regarding to the reference genome of B73. We genotyped these PAV on a collection of 480 lines representative of maize genetic diversity. A new Affymetrix pipeline was developed to call presence or absence of a genomic sequence from fluorescence intensity and contrast between probes. We evaluated the quality of the calling by comparing the expected allele from resequencing data of the 4 lines used for PAV discovery to the observed one from the array. We showed that 72% of the probes gave consistent results. PAV genotyping was used to analyze the genetic diversity within a core panel of 20 inbred lines. We showed that PAV well separated these lines according to their genetic group origin.
These results illustrate the ability of our high throughput genotyping PAV array to genotype accurately a large number of PAV and their breakpoints on a large and diverse set of maize inbred lines.

Funding acknowledgement: French National Research Agency, Amaizing Project, CNV-Maize Project

P171

Development of late temperate maternal haploid inducers

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In recent years, in vivo doubled haploid technique has been widely used on advanced maize breeding programs. Obtaining doubled haploids by in vivo maternal haploid technique shorten time of breeding and increase the efficiency of maize breeding. According to literatures, inducer lines are not available the large number in the world. In our country, maize breeders in both public and private sectors have to buy inducer lines from abroad for implementing in vivo maternal haploid technique. Most of inducer lines were used in in vivo haploid technique have adapted of the temperate zone. These inducer lines are earlier (FAO 400-450), short plant height, poor pollen yield than our maize materials and in terms of their other morphological characteristics are weaker. The breeding programs have been initiated in order to transfer from early inducer lines haploid induction features and R1-nj marker to local inbred maize lines on Maize Research Institute (MRI) in 2011. Crosses were made between three local inbred lines from MRI as female parents and inducer lines RWS, RWK-76 and inducer hybrid RWSxRWK-76 as pollinators. F2 populations were obtained in consequence of selected criterias such as anthocyanin coloration, tassel length, branch number, plant height, days to flowering and embryo-endosperm colorfulness. F3 generations were planted by ear-to-row and were selected according to late flowering, good plant vigor, high pollen yield. At the same time, every generation were crossed with liguleless line as female and were determined haploid induction rate of candidate inducer lines on F3-F7. The morphological and genetic similarity was examined between the candidate inducer lines and RWS, RWK-76 donor lines.

Funding acknowledgement: National Science Foundation (NSF), The Scientific and Technological Research Council of Turkey

P172

Developmental and morphological changes associated to flowering time shifts produced during divergent selection experiments in maize : developmental transitions and architecture

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An original plant material resulting from two Divergent Selection Experiments (DSEs) for flowering time of maize was developped over the last 20 years. Within two maize inbred lines, Early- and Late-flowering populations, subsequently structured into families within populations were formed. Comparisons between Early and Late populations or between Early or Late families within each DSE allow to study the effects of flowering time shifts in the same genetic background.
A strong response to selection was observed over the 20 generations and results indicate that this response is due to a variety of changes affecting different aspects of the life cycle. To better understand these changes, we set up field experiments to describe plant growth and development for different families issued from the DSEs. We observed that differences between Early and Late progenitors concerned timing of transitions, phyllochron, and the delay between the end of leaf emergence and blooming, while organs' growth rates were much less variable. Marked differences in developmental timings were associated with large morphological changes. Changes in the duration of developmental phases impact both plant architecture (leaf number and shape) and the duration of organ growth, and can explain changes in morphology like leaf length.
When comparing Early populations together, or Late populations together, we found that a delay in flowering time can result from changes either in the length of juvenile phase, the time of floral transition or the length of the reproductive phase. Phenotypic convergence for flowering time was therefore achieved through different developmental routes.

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P173

Developmental and morphological changes associated to flowering time shifts produced during divergent selection experiments in maize : phyllochron

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An original plant material resulting from two Divergent Selection Experiments (DSEs) for flowering time of maize was developped over the last 20 years. Within two maize inbred lines, Early- and Late-flowering populations, subsequently structured into families within populations were formed. Comparisons between Early and Late populations or between Early or Late families within each DSE allow to study the effects of flowering time shifts in the same genetic background.
A strong response to selection was observed over the 20 generations and results indicate that this response is due to a variety of changes affecting different aspects of the life cycle. To better understand these changes, we set up field experiments to describe plant growth and development for different families issued from the DSEs (phyllochron, growth rates, plant architecture, developmental transitions). We observed that differences between Early and Late progenitors concern timing of transitions, phyllochron, and the delay between the end of leaf emergence and blooming, while organs' growth rates were much less variable. Comparisons of the different families suggest that phenotypic convergence for flowering time between Early or between Late progenitors is achieved through different developmental routes.
This poster focuses on changes observed in leaf emergence rates (phyllochron) for Early and Late genotypes. We found that average values of leaf or collar emergence rates exhibited few differences between genotypes, but strong year effects. Observed flowering time shifts were therefore not associated with major changes in the phyllochron. Analysis of temporal variation of departures to average values throughout the season revealed the same non linear patterns for all genotypes, indicating that phyllochron was not constant through time. We discuss the role of different environmental factors in the temporal changes of plant growth rates.

Funding acknowledgement: INRA, CNRS, Labex BASC

P174

Dissecting the genetics of cold tolerance in maize

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Cold tolerance is a complex trait that limits adaptation of maize in temperate areas. The available literature shows that cold tolerance is regulated by large number of QTLs with small effects and weak consistence across genotypes. However, previous studies have involved a limited number of genotypes with narrow genetic diversity, and scarce genetic recombination mapping populations. We investigated the genetics underlying cold tolerance in a multi-parent advanced generation inter-cross (MAGIC) population genotyped using genotyping by sequencing (GBS) with near one million SNPs. This MAGIC population consisted on recombinant inbred lines (RILs) released from a synthetic population (EPS21) obtained from Spanish, Italian, and French flints, and two non-Reid Corn Belt inbred lines. A set of 406 RILs was evaluated in a growth chamber under cold and control conditions, as well as in the field at early and late sowing dates. Our results showed no evidence for any single large-effect quantitative trait loci (QTLs); however, a region on chromosome 2 had a dense concentration of significant QTLs for fluorescence (Fv/Fm) that were consistent in growth chamber and early field sowing. Besides, we identified numerous additional small-effect QTLs for cold tolerance-related traits in growth chamber (64 QTLs) and early field sowing (61 QTLs). The large number of markers involved, the small effects explained by each QTL, and the difficulties on collecting phenotypic data, encourage the implementation of genomic selection programs to improve cold tolerance in maize.

P175

Dissecting the role of lncRNA during maze domestication

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Long non-coding RNA (lncRNA) plays important roles in various biological processes and contributes to plant adaptation. To dissect the role of lncRNA during maize domestication, we analyzed the genome-wide *cis* and *trans* regulatory difference between maize and teosinte using RNA-seq data of F1 hybrids and parents for leaf, ear, stem tissues. We identified 1055, 1153 and 1156 *cis*-regulated lncRNAs, and 1365, 1753 and 1030 *trans*-regulated lncRNAs for leaf, ear and seed tissues, respectively. For more than 70% of *cis*-regulated lncRNAs, maize allele is more highly expressed than teosinte allele, suggesting domestication more frequently favors up-regulated lncRNAs. Furthermore, we found that *cis*-regulated lncRNAs tended to be neighbor of genes showing expression divergence between maize and teosinte especially for genes under selection. These results indicated that the regulatory differences in lncRNA might play an important role in driving the transcriptome divergence during maize domestication.

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P176

Diversity analysis within a collection of 1191 flint maize inbred lines using genotyping-by-sequencing

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Genotyping-by-sequencing (GBS) is a highly cost-effective procedure that permits the analysis of large collections of inbred lines. We used it to characterize diversity in 1191 maize flint inbred lines from the INRA collection, the European Cornfed-Flint association panel, and flint lines recently derived from landraces. We analyzed the properties of GBS data obtained with different imputation methods, by comparison with a 50K SNP array. We identified 7 ancestral groups within the Flint collection (five typically flint: Northern Flint, Italy, Pyrenees-Galicia, Argentina, Lacaune, and also Dent and Pop corn) that are in agreement with breeding knowledge. This analysis highlighted that many lines are issued from crosses between different flint ancestral groups (admixture). Approximately 200 lines also appear to be issued from crosses with dent germplasm aiming at the improvement of flint germplasm. We performed association studies on different agronomic traits, revealing SNPs associated with cob color, kernel color, and male flowering time variation. We analyzed the relationship between the haplotype diversity and the trait variation at some strong association peaks.

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P177

Diversity of maize landraces from south-west of France: origin and morphological differentiation analyzes

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In south-west of France, maize landraces had evolved under environmental condition and human management since 17th century and until the arrival of the hybrids in 1960s. In the sixties, these landraces have been conserved ex situ at the maize biological resource center (INRA, Mauguio, France).
Previous genetic studies of a sample of this collection allowed identifying a distinct genetic group named Pyrenees-Galicia. This group has been hypothesized to come from an hybridization between the Northern Flint group and the Caribbean group
In this study, we analysed a broader sample of the INRA collection in order to determine their genetic structure and to describe their morphological diversity.
Firstly, we analysed genetic diversity of 194 maize landraces from south-west of France with the 50K SNPs array and using a bulk DNA sample of 15 plants. A non-supervised admixture analysis was performed by adding 148 American and European landraces. This analysis shown that there were 8 genetic groups. In fact, two separate genetic groups were identified in South-west of France, one in the Western part and one in the Eastern part.
Secondly, we assessed morphological differentiation between the two genetic groups found in south-west of France, using a principal component analysis and an analysis of variance on 15 traits. Landraces located in West part of south-western France are earlier with bigger kernels and ears with a lower number of rows than landraces located in East part.
Finally, we performed a principal coordinate analysis (PCoA) on 194 maize landraces from south-west of France. We showed that landraces were distributed continuously along the first component of PCoA analysis. This component was correlated with geographical coordinates of the landrace collection sites, highlighting a longitudinal gradient and a latitudinal gradient.

Funding acknowledgement: ANR France : Amaizing, WAAPP (ISRA Senegal)

P178

Does subspecific variation correspond to genetic or cytotypic variation in the widespread taxon Phlox speciosa (Polemoniaceae)?

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Phlox speciosa, or showy phlox, ranges from the Sierra Nevada of California into the Coast and Cascade Ranges of the Pacific Northwest (to British Columbia), and into the Rocky Mountains in Idaho and western Montana. It grows at low to middle elevations (100-2400 m), in rocky, wooded slopes and sagebrush habitat, and is easily distinguishable from congeneric taxa by its upright habit, showy flowers, and short length of the style relative to the stigmas. Several subspecies and varieties were identified by previous taxonomists (originally Edgar Wherry in 1955), based on the obvious morphological variation in the group, but this variation does not correspond well to geography, and the current Flora of North America taxonomic treatment has suspended the recognition of subspecific taxa in P. speciosa pending genetic and cytotypic investigation. We are exploring the genetic diversity and connectivity of 25 populations from across the range of this species, to test the hypothesis that the observed patterns of morphological and ecological differentiation between populations are due to genetic discontinuity rather than simply phenotypic plasticity. Field sampling of leaf tissue (20 plants per population) was guided by our study of previously collected herbarium specimens. Thus far, our flow cytometry results show little cytotypic variation in the species: out of 21 populations sampled to date, all proved to be diploid (2n = 14) except one tetraploid population. We can conclude that the phenotypic and habitat variability that P. speciosa exhibits is not due to ploidy-level differences leading to intraspecific reproductive isolation. This finding has provided the basis for our exploration of genetic connectivity between populations using codominant genotyping data from seven microsatellite markers designed specifically for western North American Phlox species. This research will inform important evolutionary questions about species limits and subspecific variation in the genus Phlox.

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P179

eQTL mapping and genome wide association study for maize leaf traits using markers derived by RNA sequencing of two RIL populations

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The capacity to generate high throughput sequencing data on genetic plant materials revolutionized the characterization of complex traits. Recently, the use of expression quantitative trait loci (eQTL) approach emerged as an effective tool for identifying genomic regions affecting gene regulation. Concurrently, genome wide association studies (GWAS) became a powerful tool to identify the genetic contribution to phenotypic outcomes. eQTL analysis combined with GWAS may provide a detailed picture of the process by which genetic variants affect complex traits. In this study we focus on 200 RILs derived from two maize populations: a B73xH99 cross and the multiparental MAGIC maize. We analyzed RNAseq data produced in proliferative leaf tissues in each of the 200 RILs to derive a variant callset for downstream analysis. The bioinformatics pipeline for variants discovery in RNAseq data followed the GATK best practices. After a stringent filtering for marker quality, we obtained several hundred thousand genome-wide SNPs. When compared to sparse molecular markers previously developed on the RIL populations, these SNPs proved solid in describing the RILs genetic makeup. The RILs were phenotyped for ten leaf traits describing the dynamics of seedling growth. We integrate the genomic, transcriptomic and phenotypic information in eQTL and GWAS approaches to identify candidate genes for early leaf traits in maize. A preliminary eQTL mapping identified more than 30,000 significant cis and trans eQTL. When used in a GWAS approach, the RNA-derived SNPs reported highly significant associations with the leaf traits. Linkage disequilibrium decay was used to group the several thousand marker-trait associations in approximately 30 QTL currently under characterization. So far, only a limited number of studies have integrated eQTL data and GWAS to investigated the plant growth mechanisms. Our approach will refine the identification of candidate genes in QTL and allow to prioritize loci relevant for breeding for early vigor.

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P180

Evaluation of functional stay-green in maize inbred lines and their relationship with agronomic traits

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The time and rate of senescence in maize affects the duration of photosynthetic activity and the remobilization of nutrients from leaves to grain which has an impact on biomass and grain yield. Various types of late senescence or stay-green have been proposed which can be reduced to two types: functional, when the photosynthesis is active while the plant is green, and cosmetic, when photosynthesis is not working out in spite of the green color of the leaves. Little information has been published about the types of stay-green in inbred lines of temperate maize. We evaluated the functional stay-green of 197 lines developed in different public and private breeding programs. Many of the lines were included in the study because they had visual stay-green according to personal information of breeders or information found in the certification of the lines. Other lines were included because of their relevance in maize breeding, for example B73, PH207, Mo17, etc. We found the reduction in chlorophyll content was always accompanied by a decrease in photosynthetic rate, although, some lines retained small amounts of chlorophyll after losing its photosynthetic activity. Therefore, we concluded that the predominant way of stay-green in inbred lines of temperate maize is functional. There was variation in the duration of photosynthetic activity between the inbred lines. Some lines had active photosynthesis as late as 75 days after flowering, while many others did not. The lines with late photosynthesis had on average the highest grain yield and biomass yield, but also the highest grain filling duration and the highest grain moisture. We conclude that the functional stay-green is favorable for grain yield, but at the cost of increase the grain moisture.

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P181

Exploring the genetic basis of cell wall traits upon contrasted water regimes in maize

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Cell wall digestibility and composition are the major targets for improving both feeding value and industrial valorizations (such as bioethanol) from lignocellulosic biomass. Biomass production should also reach expected yields under environmental-friendly practices. It was brought back that the variations of the biomass quality and composition are not only impacted by genetic, but also by environmental factors, such as water stress episodes. To guide breeding of maize and other dedicated C4 species for biomass production, we evaluated a F271 x Cm484 recombinant inbred population under non-irrigated and irrigated conditions during three consecutive years near in Montpellier (South of France). We quantified over 1,300 harvested stover samples using dedicated near-infrared spectroscopy equations established with calibrated samples harvested under both water regime conditions. We showed that biomass digestibility and composition varied between irrigated and non-irrigated scenarios. Using a genotyping-by-sequencing approach, we then built a dense genetic map with 1,000 single nucleotide polymorphism (SNP) markers and performed single-marker analyses to identify constitutive quantitative trait loci (QTLs) across years and conditions, and responsive QTLs using the interaction effect between the marker and the treatment. Overall, we identified 16 clusters of constitutive QTLs and 5 clusters of responsive QTLs, of which only one did not co-localized with constitutive QTLs. These results showed that co-localization between traits were different depending on the QTLs, underlying different strategies for breeding.

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P182

Fine mapping of metabolite-QTLs for extracellular surface lipid accumulation on maize silks

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Upon emergence from the husk leaves, maize silks are exposed to numerous environmental stresses (e.g., UV radiation, insect damage, and desiccation). Like most other aerial plant surfaces, the maize silk has a cuticle infused with and coated by extracellular surface lipids (SLs) that act as an environmental barrier. The silk SL metabolome includes at least 50 metabolites that are primarily linear hydrocarbons, fatty acids, and aldehydes ranging in chain lengths from 16 to 35 carbon atoms. To identify the genomic loci controlling the biosynthesis of these metabolites, we performed metabolite-quantitative trait locus (mQTL) mapping using the intermated B73xMo17 recombinant inbred line (IBMRIL) population, which harbors considerable variation in the silk SL metabolome. Surface lipids were extracted from emerged silks at three days post-silk emergence and subsequently identified and quantified using gas chromatography-mass spectrometry (GC-MS) or GC-flame ionization detection (GC-FID). mQTL analysis of constituent traits, metabolite-class traits, and relative composition traits identified >500 mQTLs that modulate the abundance and composition of the silk SL metabolome, with some mQTLs detected in more than one environment. A more complete characterization of the genetic network has been pursued through inclusion of traits that are precursor (fatty acids), proposed intermediate (aldehydes), and end-product (hydrocarbons) lipids. To connect this genetic network to the predicted biochemical network for hydrocarbon biosynthesis, identification of causal genetic polymorphisms or the ability to discriminate among competing candidate gene hypotheses is required. Here we report our progress in dissecting two genomic loci that are particularly influential in shaping the silk SL metabolome. Fine mapping results are reported from three complementary breeding and analysis approaches: isogenic dual testcross, heterozygous inbred family and bi-parental introgression, each of which are used to interrogate potentially informative recombination events.

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P183

Flowering time stability in doubled haploid lines derived from exotic maize

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Variation in flowering time phenotypes across maize germplasm is effected by a combination of the plant's genotype, environment, and genotype-by-environment interaction. When adapting exotic germplasm to the United States Corn-Belt, the plant's genotype is the crucial hindrance as it controls fundamental adaptation traits such as photoperiod sensitivity and overall flowering time. The unknown effect of the new environment on the germplasm's genotype exacerbates this issue. The phenotypic plasticity created is ill-defined if moving specific exotic germplasm over large latitudinal distances and for the adapted variants being created. Reduced plasticity, or stability, is desired for the adapted variants as it allows for a more rapid implementation into breeding programs throughout the Corn-Belt. Herein, doubled haploid lines derived from exotic maize, developed in a joint venture between the USDA Germplasm Enhancement of Maize project and Iowa State University and adapted through backcrossing exotic germplasm to elite, adapted lines, are studied for flowering time stability. Reaction norms are used to visualize patterns across multiple environments and to quantify stability. Genome-wide association studies are used to further explore and dissect genes and pathways. Knowledge gained will be implemented for adapting additional exotic germplasm.

Funding acknowledgement: United States Department of Agriculture (USDA)

P184

From the sequencing of 16 MAGIC population founders to a 8 million SNP resource on the BALANCE panel

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Association studies have become a method of choice to identify genomic regions involved in the variation of traits of interest, especially in maize. BIOGEMMA has created a panel of DH lines from a MAGIC population developped from 16 founder lines. This panel has suitable properties to map QTL at a high resolution provided that a wealth of markers evenly spaced across the genome is available. To significantly increase the number of markers in the DH lines:
- the founder lines were fully sequenced through Illumina HiSeq technology with a 15X mean coverage (mapping on B73 RefGenV2),
- DH lines together with the founder lines were genotyped with the Axiom maize 600K chip and the genome of each DH line was reconstructed as a mosaic of the founders' genomes using statistical methods imported from mouse studies,
- the founder sequence data were projected onto the each DH genome using a home-made R program.
More than 12 million of SNP were detected from the sequence data among which 8 million were selected using different selection criteria (allelic segregation, IBD, LD). This extensive genotyping dataset represents a powerful genetic resource to decipher genetic bases of complex traits especially when combined with all the other "omics' layers aggregated in this panel.

P185

Genetic and molecular basis of maize hybrid vigor: genome wide association studies in factorial hybrid designs

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A common strategy to detect QTL for hybrid performance is to cross a population of inbred lines from one heterotic group to a same common parent derived from the other heterotic group, referred as "tester". In the context of linkage based mapping, the use of a tester raises important issues such as dependency of the results according to the tester and buffered genetic variation. Also, analyzing two genetic groups calls for two experiments. Giraud et al. (2017, Genetics) have therefore advocated the study of both heterotic groups at a same time.
During the PIA/ANR Amaizing project, we assembled a panel of hybrids between the two complementary groups largely used for maize production in northern Europe. Around 300 dent and 3oo flint lines were crossed according to an incomplete factorial design to produce 348 hybrids. The hybrids were evaluated in 8 environments for yield components and phenology. Lines were genotyped using 600K SNP markers chip. Missing data were imputed using Beagle and markers with a minor allele frequency lower than 4% were discarded. Following a classical approach (Technow et al., 2014), the data were analyzed by decomposition into the general combining ability (GCA) of each group and the specific combining ability (SCA) between groups (Sprague & Tatum, 1942). The results showed that the contribution of GCA is largely more important than that of SCA. The QTL detection implied to take into account the relatedness within and between the two parental populations. Different hypotheses for the QTL effects were tested (additive, interaction and global marker effects) either environment by environment and for all the environments taken together. The results indicated that i) the associations that are detected differ according to effects included in the model and ii) the number of QTLs detected in the multiple environment context is higher than in individual environments, confirming the interest of such multi-environment analysis.

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P186

Genetic architecture of complex traits in a maize-teosinte population

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Domestication and improvement have profoundly altered wild species to meet human needs. Maize has undergone a striking transformation from the wild progenitor, *Zea mays* ssp. *parviglumis*, resulting in great morphological differences. In this study, a Mo17-mexicana BC2F5 population of 191 families was developed to dissect the genetic architecture of 18 divergent morphological traits, including 2 plant, 3 flowering, 6 ear, 2 tassel and 5 seed traits. Totally, 109 individual QTLs, which were clustered into 67 loci, were identified for 18 traits, with each QTL explaining 3.4%-29.4% of phenotypic variation. For each trait, the number of identified QTL ranged from 3 to 12, and the totally explained phenotypic variation ranged from 28.0% to 77.8%. In addition, we identified 4 pairs of epistatic QTL, accounting for a small proportion of phenotypic variation for 4 detected traits. These findings indicate that the additive effects play more important roles than epistatic effects in the genetic architecture of the divergent morphological traits in the maize-teosinte population. Among 109 identified QTLs, 55 and 28 QTLs underwent domestication and improvement, respectively. A QTL-trait network constructed based on the identified individual QTL revealed 66 QTLs with pleiotropy effect, which has the same positive or negative additive effects for two or more traits. Our results provide useful information for future research on the genetic basis of these traits.

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P187

Genetic dissection of plant height and flowering using two Stiff Stalk multi-parent advanced generation intercross populations of maize

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Multi-parent advanced generation intercross (MAGIC) populations can be used to elucidate the genetic control of complex traits in plants. Two MAGIC populations were developed at the University of Wisconsin from six inbred parents (PHJ40, PHB47, LH145, NKH8431, B84, and B73) belonging to the Stiff Stalk heterotic pool. Parents were crossed in all possible F1 combinations, and following balanced intermating of the F1 progeny, the populations underwent additional intermating before doubled haploid (DH) generation. Version 1 (V1) of the population had two generations and Version 2 (V2) had four generations of random intermating. The goal of this project is to compare the level of variation present in the two populations and to determine differences in map size and power to detect genotype-phenotype association in these MAGIC populations. Approximately 360 V1 and 430 V2 DH lines were grown along with the six parents as checks in two field replicates in 2016 and 2017 in Wisconsin. Growing degree days (GDD) until anthesis and silking was evaluated on a whole-plot basis, while three representative plants were evaluated for plant height, ear height, and cob color. Across both years, V1 had an average plant height of 188 cm, SD 19.5 cm, while V2 had an average plant height of 182 cm, SD 21.3 cm. V1 had later anthesis with an average of 1366 GDD, SD 60.1 GDD, while V2 had an average of 1354 GDD, SD 57.8 GDD. V1 also had later silking with an average of 1382 GDD, SD 65.1 GDD, while V2 had 1375 GDD, SD 62.6 GDD. A genetic map was developed using R/qtl2 (kbroman.org/qtl2) and markers scored from exome capture sequencing. Outcomes from this research will further our understanding of the utility of different structures of MAGIC populations for QTL detection.

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P188

Genetic distance in relation with specific combining abilities and heterosis for vegetative traits in maize

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Prediction of maize hybrids performances based on the genetic distance between their parents has long been an important path in the implementation of molecular marker technology in maize breeding programs. In this study genetic distance assessment of six maize inbred lines was done using 21 SSR primers. In the analysis, 92 alleles were detected with a mean of 4.38 per locus. Genetic distance (GD) determined using Simple matching coefficient (SM) between inbred lines ranged from 0.11 to 0.549. Using UPGMA clustering method a dendrogram was constructed in wich inbred lines were separated into two main cluster, one containing 2 inbred lines, and the other one with 4 inbreds. The bigger cluster furthermore was divided into two smaller subclusters. This classification was in accordance with known pedigree data of analysed genotypes. Six investigated inbred lines were furthermore crossed according to an incomplete diallel design forming 15 hybrid combinations. These genotypes were tested in field trials on 3 locations together with inbred lines per se. In this study maize vegetative traits: plant height and leaf number per plant were analyzed. Statistically significant specific combining abilities (SCA) and high parent heterosis (HPH) were calculated for both traits. Spearman's rank correlation coefficient between genetic distance and SCA for plant height and leaf number was positive and statistically significant while the correlation between high parent heterosis and genetic distance although positive was not significant.

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P189

Genetic variation for early development and cold tolerance in DH libraries from maize landraces

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Due to the high sensitivity of current maize varieties to cold stress in early development, farmers bear the risk of yield losses because of cold periods in late spring. Improving cold tolerance in maize could reduce this risk and would allow earlier sowing dates. The European maize landraces are considered as "gold reserve" of genetic variation for many quantitative traits. Strategies for efficiently utilizing this resource are currently evolving. In our study, we aim to detect relevant regions for the expression of cold tolerance through genome-wide association studies (GWAS). Further, we examine the practicability of whole-genome based prediction of genotypic values in material derived from landraces with a focus on cold tolerance related traits. We applied in-vivo doubled-haploid (DH) induction to three selected landrace populations and produced libraries of about 1000 DH lines. The complete set of DH lines was genotyped using the 600k Affymetrix® Axiom® Maize array. In 2017, all lines were phenotyped for early vigor, early plant height, flowering time, final plant height and other agronomic traits in six diverse environments in Germany and Spain.
In GWAS, we identified genomic regions significantly associated with cold tolerance related traits on chromosomes 1 and 10, respectively. Cross-validated genomic predictions yielded intermediate to high predictive abilities for cold tolerance traits within landrace populations (0.48 to 0.60). In predictions across landrace populations, predictive abilities were close to zero. We will use these resources to identify novel candidate genes and to assist breeding for cold tolerance in elite germplasm.

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P190

Genome wide association studies for kernel starch and protein content in the Wisconsin diversity (WiDiv) maize association panel

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In maize forage production, the kernel is a major contributor of energy, therefore improved grain starch content and bioavailability for digestion are important plant breeding objectives. Endosperm texture affects starch degradation rates in ruminants. The range extends from loosely packed (floury) to densely packed (vitreous) starch granules types. This condition is associated with the amount and type of proteins found in the matrix that surrounds the starch granules. The main storage proteins in the endosperm are prolamins, also known as zeins. The main objective of this study is to explore the natural variability of kernel starch and protein content of the maize Wisconsin Diversity (WiDiV) association panel and identify region of the genome associated with these traits. This population is genotyped with 899,784 RNA-seq based single nucleotide polymorphism (SNP) markers. Open pollinated plants were grown in a randomized complete block design with two replicates in Arlington, WI in the summer of 2013 at a density of approximately 65,000 plants/ha. Ears from three representative plants per plot were collected after plants had reached physiological maturity. Kernels from the set of three ears from 561 of the WiDiv lines were scanned using Near Infrared Transmittance (NIT) to obtain grain starch and protein percentage. Significant variation was found for starch (40% to 75% of grain biomass) and total protein (6% to 13.5% of grain biomass) content among genotypes. Repeatabilities of 0.85 and 0.9 were observed for starch and protein content, respectively. We found one significant SNP on the long arm of chromosome 1 associated with kernel starch content. No significant associations were found for total protein content. Investigating regions of the genome associated with starch and protein content could open new opportunities to understand the contribution of these regions to grain quality in the context of forage maize.

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P191

Genome wide association study for protein expression under normal and water deficit conditions in maize leaves

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Maize is one of the main crops worldwide, but its yield can be severely affected by drought. Drought tolerance in maize is genotype-dependent, indicating that this trait can be genetically improved. This study aimed to identify genetic determinants of drought tolerance by using genome wide association study (GWAS) to identify QTLs for protein abundance (pQTLs) that colocalize with QTL for ecophysiological traits. A panel of 251 maize genotypes was grown in the high-throughput phenotyping platform PhenoArch (Montpellier) under water deficit (WW) and well-watered (WD) conditions and in two replicates. Several ecophysiological parameters were measured during plant growth and analyzed elsewhere (Alvarez Prado et al., Plant Cell Environ. 2017; 1-13). At the pre-flowering stage, 1004 samples were taken on the last ligulated leaf and analyzed by shotgun proteomics. A total of 1950 proteins were quantified. Most of them showed significant abundance variations in response to water deficit and to genotype. In particular, drought responsive proteins, like dehydrins, were highly induced under WD, while proteins of energy metabolism were down-regulated. GWAS was performed on 3900 molecular phenotypes (=1950 proteins x2 conditions). 29004 pQTLs were detected for 3758 (96.4%) molecular phenotypes, confirming the high genetic variability of protein abundances. Local pQTLs, located <1M pb from the protein encoding gene, had much stronger effect than distant pQTL (19.3 % vs 5.5 % of explained variance on average). More than 30 cases of pQTL/QTL colocalizations involving ecophysiological traits such as water use or transpiration were detected, mostly in WD. Several candidate genes potentially controlling both the abundance of a protein and a phenotypic trait were identified. Altogether, these results show the potential of high-throughput quantitative proteomics to decipher the determinisms of protein abundance regulation and to discover candidate genes and proteins potentially involved in the variation of plant phenotypic traits.

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P192

Genome-Wide association of biomass digestibility in the Wisconsin diversity panel

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Maize stover comprises all the non-grain portions of the plant and accounts for approximately half the total biomass yield. The stalk contributes most to stover biomass, and due primarily to secondary cell wall formation during growth and development, it is recalcitrant to biological degradation. In the context of forage production for ruminant nutrition and biofuel production, compositional attributes which reduce recalcitrance are of interest. In this experiment, we collected the second lowermost stalk internode of ~4700 plants 45 days after flowering and evaluated them for compositional characteristics by near infrared spectroscopy (NIRs). These plants represent 667 diverse inbred lines from the Wisconsin Diversity (WiDiv) association panel, which were evaluated in a replicated field trial across two years. Prediction equations were developed for neutral detergent fiber (NDF) and acid detergent fiber (ADF) of stalk internodes. NDF represents total cell wall concentration (hemicellulose, cellulose, lignin) while ADF encompasses cellulose and lignin. Prediction accuracies for NDF and ADF using NIR were 0.95 and 0.94, respectively. Hemicellulose content was calculated from the percentage of total cell wall constituents. Significant genetic variation for hemicellulose was observed for the collection of lines, with values ranging from 16.9 to 36.1% of the dry matter. Hemicellulose was used to conduct a genome wide association analysis using 899,784 SNPs generated by RNA-sequencing. This study demonstrates the utility of the WiDiv association panel coupled with a dense marker set, to identify genomic regions associated with biomass digestibility and more efficiently aid the development of superior forage maize varieties.

Funding acknowledgement: National Science Foundation (NSF)

P193

Genome-wide mapping of kernel color in maize (Zea Mays L.) reveals associations with isoprenoid and carotenoid biosynthesis genes and carotenoid degradation genes

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Rapid development of biofortified crop varieties with improved levels of provitaminA and total carotenoids would benefit from a greater understanding of relevant and useful genetic variation in the carotenoid and isoprenoid biosynthetic pathways, and carotenoid degradation enzymes. Higher levels of provitamin A (proVA) and total carotenoids in grains, roots, tubers and fruits are frequently accompanied by an increase in orange color. Orange color in maize endosperm is being used successfully with consumers in Africa to differentiate the higher proVA varieties. Genetic loci affecting maize kernel color were revealed through genome-wide association study (GWAS) in a large association panel. Variation in the dxs2 and psy1 genes, both expressed in maize endosperm and the first committed steps in the plastidic synthesis of isoprenoids and carotenoids, respectively, were significantly associated with quantitative measures of kernel color determined by colorimeter analysis. The lcyE gene, which affects partitioning between the α- and β-branches of the carotenoid pathway, and zep1, which affects flux out of the β-branch of the carotenoid pathway, were also associated with color values in GWAS. Because the β-branch carotenoids are more orange in color than more yellow α-branch carotenoids, a relative increase in β-carotenoids relative to α-carotenoids will alter color. This shift is desirable because the major proVA compounds present in maize, β-carotene and β-cryptoxanthin are part of the β-branch. The performance of isoprenoid and carotenoid pathway analysis revealed an association with color of dmes2, a gene involved in isoprenoid synthesis. Our results show that there are commonalities between the genes influencing color revealed in this study and genes influencing levels of proVA and total carotenoids reported in other studies. These results establish colorimeter as a quantitative means of relating color to total carotenoids.

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P194

Genome-wide SNP genotyping of DNA pools identifies original landraces to enrich maize breeding pools

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Maize landraces germplasm have a very large genetic diversity that is still poorly characterized and exploited in plant breeding programs. We studied the effect of both human selection and environmental adaptation on genome-wide diversity of landraces with a focus on landraces-hybrid transition in order to identify interesting source of genetic diversity to enlarge modern breeding pools. We developed a high-throughput, cheap and labor saving DNA pooling approach based on 50K SNP maize Illumina array and estimated thereby allelic frequencies of 23412 SNP in 156 landraces representing worldwide maize diversity. We compared the diversity of this collection at genome-wide scale level with that of a panel of 336 inbred lines. Our new approach: (i) gives accurate allelic frequencies estimation that are reproducible across laboratories, (ii) protects both against false detection of allele presence within landraces and against ascertainment bias. Modified Roger's genetic Distance estimated from 23412 SNP and 17 SSR on same DNA pool are highly correlated, which validates our approach. Accordingly, structuration analysis based on SNP gives consistent results with SSR for higher levels of structuration but gives a slightly different pictures for more advanced structuration levels, suggesting that SNP and SSR could capture differently recent evolution. Gene diversity of landraces varies strongly along the genome and according to geographic origins. We identified 376 SNP under diversifying selection unraveling a selective footprints in *Tga1/Su1* regions. While some maize landraces were closely related to several inbred lines and strongly contributed to modern breeding pools as Reid Yellow Dent or Lancaster Surecrop, some other have no related inbred lines and seem to have poorly contributed. We identified limited diversity loss or selective sweep between landraces and inbred lines, excepted in centromeric regions. For these regions, original landraces could be interesting to enlarge genetic diversity of modern breeding pools.

Gene / Gene Models described: *Tga1 (teosinte glume architecture1), Su1 (Sugary1)*; GRMZM2G101511, GRMZM2G138060
Funding acknowledgement: Agence Nationale de la Recherche (ANR), Association pour l'étude du mais (Promais)

P195

Genomic prediction within and among doubled-haploid libraries from maize landraces

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Thousands of maize landraces are stored in seed banks worldwide. Doubled-haploid libraries (DHL) produced from landraces allow to harness their rich genetic diversity for future breeding. We investigated the prospects of genomic prediction (GP) for line per se performance in DHL from six European landraces (CG, GB, RT, SF, SM and WA) and 53 elite flint (EF) lines by comparing four scenarios: GP within libraries (1L) as well as incrementing training set (TS) size, between pairs of libraries (LwL), and among combined libraries, either including (LwCLi) or excluding (LwCLe) lines from the TS that belong to the same DHL as the prediction set. GP was performed using the GBLUP model and we report the prediction accuracy (ρ) averaged across seven agronomic traits.
We will compare the different validation scenarios to investigate if GP can benefit from the information of several landraces. Factors affecting ρ will be evaluated, such as training set size, linkage disequilibrium (LD) and expected degree of relatedness between genotypes. The expenditures to create DHL are high, thus, we will give recommendations regarding which factors to focus on when using GP to harness the genetic variability of landraces.
Literature: http://www.europeanmaize.net/

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P196

Genomic regions associated with Goss's Wilt resistance in the commercial maize germplasm pool.

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Goss's Wilt (Clavibacter michiganensis subsp. nebraskensis) is a bacterial disease of maize (Zea mays) that manifests itself predominately as a leaf blight. Recently, it has spread from the high plains of the United States and is considered an emerging threat to Ontario. Fungicides are ineffective at controlling Goss's Wilt; however, development of resistant hybrids may be an option for mitigating yield losses. Current varieties grown in Canada appear to have little or limited resistance to this pathogen. Resistance is thought to be quantitative and controlled by 9-11 QTLs, making it more difficult to select for compared to single gene resistance. The objective of this study is to use in silico mapping using a North Carolina Design II mating scheme to identify genomic regions associated with Goss's wilt resistance in 20 stiff stalk and 30 non-stiff stalk inbred lines from the modern Canadian commercial germplasm pool and 600 hybrids derived from inbred crosses. QTL mapping will be conducted on the hybrids and molecular markers will be developed. The proposed research will result in the identification of genomic regions in the modern commercial germplasm pool utilized by breeding companies, thereby facilitating rapid utilization of resistant material in commercial maize breeding programs.

P197

Genomic selection efficiency and a priori estimation of accuracy in a structured dent maize panel

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Key message: Population structure affects genomic selection efficiency as well as the ability to anticipate accuracy using standard GBLUP.

Genomic selection refers to the use of a prediction model calibrated on a set of individuals to predict the genetic value of new individuals using marker data. Prediction models usually assume that the individuals used to calibrate the prediction model belong to the same population as those to be predicted. Most of the a priori indicators of precision, such as the Coefficient of Determination (CD), were derived from those same models. But genetic structure is a common feature in plant species and it may impact genomic selection efficiency as well as the ability to anticipate prediction accuracy. We investigated the impact of genetic structure in a dent maize panel ("Amaizing Dent") using different scenarios including within or across group predictions. For a given training set size, the best accuracies were achieved when predicting individuals using a model calibrated on the same genetic group. Nevertheless diverse training panels representing all subgroups also appeared efficient and should be recommended when the target population is not yet determined. Alternative prediction models, taking genetic structure explicitly into account, did not allow us to improve the prediction accuracy compared to GBLUP. We also investigated the ability of different indicators of precision to anticipate accuracy in the structure based scenarios. There was a global encouraging trend of the CD to differentiate scenarios, although there were specific combinations of target populations and traits where the efficiency of this indicator proved to be null.

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P198

Genomic signatures of local adaptation unveil association with present phenotypic variation in teosintes

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Teosintes are maize's wild relatives and can be found in a vast range of environmental conditions. During maize domestication and posterior breeding, a substantial amount of genetic variation was lost through consecutive bottlenecks. Contemporary wild teosinte populations harbor rich genetic variation, some of which is expected to be adaptive. Teosintes display pronounced population structure and phenotypic differences, which adds to their attractiveness for the study of local adaptation and underlying adaptive variation. We have chosen to study the two subspecies closest to maize (*Zea mays* ssp. *parviglumis* and ssp. *mexicana*) that are found only in Mexico and occupy different environmental niches. From a sampling of 37 populations along two altitudinal gradients, we sequenced the genomes of 6 environmentally extreme populations. Following a reverse ecology approach, genomic data was screened for Single Nucleotide Polymorphisms (SNPs) with high differentiation between lowlands and highlands and/or displaying strong correlation with environmental variables. The resulting 218 candidate SNPs were further genotyped on all 37 populations as well as on an association mapping panel composed of 11 of the 37 populations. This panel was grown in a two-year two-location common garden experiment at mid-altitude where plants were scored for 18 phenotypic traits. After accounting for neutral genetic structure by genotyping 35 microsatellite markers, we found that ~50% of our candidates are associated to at least one trait. We also observed that candidate selection methods have a considerable influence in delivering significant associations. Traits such as female and male flowering time and the number of lateral branches, presented an enrichment of associated SNPs and followed a phenotypic distribution consistent with spatially varying selection. Throughout this research we have successfully applied population genomic methods to uncover genetic variants with a phenotypic link that are segregating in natural populations.

P199

Genotyping-by-sequencing highlights original diversity patterns within a European collection of 1191 maize flint lines, as compared to the maize USDA genebank

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Comparing and identifying interesting sources of genetic diversity that have been maintained by different genebanks and understanding the global organization of this genetic diversity is an important issue for pre-breeding. We aimed at evaluating how genotyping-by-sequencing (GBS) technologies can address these both issues. We used GBS to compare the genetic diversity of 1191 European Flint lines maintained by INRA and other European institutes (see Gouesnard et al., this meeting) with the USDA collection (Romay et al., 2013, Genome Biology).
We first examined the similarity of 68 inbred lines with a same variety name between the two collections, and observed that IBS ranged from 0.775 to 0.997 (with a mean of 0.941). It indicated that GBS can be used for comparing collections and identifying redundancy and illegitimate accessions between genebanks. Based on principal coordinate analysis and structure analysis on 4001 lines, we showed the distinctiveness of flint materials compared to the USDA collection. The structuration analysis in 12 groups confirmed the influence of some historical founder lines in the genetic organization of the dent group (B73, A632, Oh43, Mo17, W182E, PH207 and Wf9). Flint lines were structured in 3 groups (a Sweet-Northern Flint group, an Italian-Argentinan group and a European group formed by Pyrenees Galicia and Lacaune groups). The Tropical and Pop corn groups were distinct. We identified several selective sweeps between Dent, Flint and Tropical inbred lines that co-localized with SNPs associated with flowering time variation identified by association mapping. It suggests that these genomic regions played an important role in adaptation to higher lattitude. The joint analysis of collections by GBS offers opportunities for a global diversity analysis of maize inbred lines.

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P200

Germplasm enhancement using former plant variety protected inbreds

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Inbreds with expired Plant Variety Protection (PVP) certificates can be a cost-effective source of elite germplasm. When developing new inbreds, the best breeding populations have both the highest mean and the highest variance. Publications exist on genomic prediction of virtual breeding populations, but none have used maize hybrid testcross data as the
training set. We predict the mean (μ) and variance (VG) of simulated breeding populations, and Identify the best parents to use in a breeding cross for inbred development. We present a phenogram of diversity of maize exPVP inbreds. We also present various quantitative genetic analyses using exPVP germplasm for identification of loci associated with grain yield, and various prediction studies.

Funding acknowledgement: Dow AgroSciences

P201

Grain yield and stability parameters of ZP maize hybrids grown in Serbia in the 2014-2017 period

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In Serbia, maize is annually sown on approximately 1 million hectares. Since Serbia is a significant producer of maize, a special attention has been paid to studies of new maize hybrids. Due to this reason, each year, the experts of the Maize Research Institute, Zemun Polje perform production trials of hybrids most distributed in the market. The aim of such trials is to obtain a detail insight not only into potential and quality of each hybrid, but also into their stability and regional distribution in the Serbian market. Ten commercial ZP hybrids of FAO 300-600 were tested in this study. The trial was carried out in 152 locations in the 2014-2017 period in the following mode: 50, 42, 42 and 18 locations in 2014, 2015, 2016, 2017, respectively. The average maize grain yields were recorded in 2014 and 2015, while 2016 was a high yield year. Drought in 2017 resulted in the yield reduction. Yield stability was estimated by the method developed by Eberhart and Russel (1966). The average four-year yield recorded in all 152 locations amounted to 8.639 t ha-1. The lowest (8.106 t ha-1), i.e. the highest (9.306 t ha-1) yield was detected in the hybrid ZP 341, i.e. ZP 606, respectively. The highest yield stability was recorded in the hybrid ZP 427 (bi=0.967). The top yielding hybrid ZP 606 also expressed high stability (bi=1.054), as well as the hybrid ZP 666 (bi=1.054). The hybrid ZP 341 (bi=0.897) was the most unstable hybrid, showing significantly better adaptation to poorer growing conditions. Hybrid ZP 560 (bi=1.102) was also unstable, but was better adapted to favourable growing conditions. Based on four-year results it can be concluded that medium early and medium late hybrids (FAO 400-600) may be recommended for the production of commercial maize in the region of Serbia.

P202

GWAS for resistance to mediterranean corn borer and agronomic traits in a MAGIC population of maize

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Understanding of the genetic basis of quantitative traits such as resistance to pest and yield are necessary to optimize the breeding programs to simultaneously improve both characters. Genome Wide Association Studies (GWAS) are useful tools to achieve this goal, especially when the mapping population is a Multi-Parent Advanced Generation Intercross (MAGIC) population. A MAGIC population of 672 recombinant inbred lines (RIL) was generated after inter-mating for six generations a synthetic variety composed of eight genetically diverse founder lines. This MAGIC population have been genotyped by genotyping based on the sequence (GBS) and evaluated for resistance to attack by the Mediterranean Corn Borer (MCB, Sesamia nonagrioides) and for yield, plant height and silking. We found seven QTL and six candidate genes for grain resistance, forty-eight QTL and sixty candidate genes for plant height, thirty-four QTL and forty-nine candidate genes for silking time, five QTL and nine genes for tunnel length, and finally fifteen QTL and twenty-four candidate genes for grain yield. We expect that tunnel length produced by MCB larvae attack and plant height also increase when yield is improved due to the genetic correlation between these traits. The intermediate role of plant height on the undesirable genetic relationship between yield and resistance was highlighted suggesting that tradeoff between plant growth and resistance could mediate that undesirable relationship. MAGIC population was useful to discover genomic regions involved in MCB resistance with greater precision than those determined using more structured populations,. The GRMZM2G178190 (Zm00001d048129) gene related to the natural resistance could intervene in the ear resistance and the GRMZM2G057140 (Zm00001d043286) gene that is related to the regeneration of the cell wall could be related to stem resistance to corn borer attacks. A genetic improvement program can be designed with the 5 QTL detected for tunnel length using markers-assisted selection to decrease the tunnel length without neglecting QTL for grain yield, thus improving both traits.

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P203

GWAS of inflorescence architecture in maize

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A number of genes have been shown to be involved in the differentiation of male and female maize inflorescences in both mutant characterizations and in bi-parental populations. Fine-mapped regions of QTL for inflorescence traits identified in the maize NAM population did not contain the genes previously assumed to underlie the QTL. This result suggests that previously unknown variation may have a significant contribution variation in inflorescence traits. This conclusion was further tested in Genome-wide association study of 21 tassel and ear traits measured in 2302 lines of the Ames association panel. A low number of significant associations were detected and these did not coincide with genes previously established to affect inflorescence architecture. Nevertheless association analysis resulted in a significant SNP identified in the coding region of Zm00001d052355, a WRKY77 transcription factor and Zm00001d046642, a GDSL esterase/lipase. While these genes have not been shown to affect inflorescence, they are likely to affect development and can be considered new inflorescence candidate genes. Together these results suggest that a potentially large number of genes of small effect, including the new candidates revealed in this study, influence inflorescence architecture on a population wide basis.

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P204

Hydrotropism: an important root trait for drought and heat avoidance in maize

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Roots of higher plants change their growth direction in response to moisture, avoiding drought and gaining maximum advantage for development. This response is termed hydrotropism. There have been few studies of root hydrotropism in grasses, particularly in maize. We developed a laboratory bioassay for testing hydrotropic response in primary roots maize elite DTMA hybrids and several maize landraces. The hydrotropic response was classified as robust or weak according to the angle of curvature, having a weak response those roots with less than 40°, and a robust response those roots with more than 40°. We recently showed the benefit of intensive phenotyping of hydrotropism in primary roots since maize plants that display a robust hydrotropic response grew better under drought and partial lateral irrigation, indicating that a selection for robust hydrotropism might be a promising breeding strategy to improve drought avoidance in maize. Robust root hydrotropic response in maize correlated with other root specific traits such as: increased lateral root branching, decrease in above ground crown roots, decreased formation of root cortical aerenchyma, increased cortical cell file number, and decreased cortical cell size. This indicates that the utility of the root hydrotropic response phene or trait depend on interactions with other root traits (phenes) in integrated phenotypes. GWAS of robust and weak hydrotropic response revealed a variety of transcription factors, proteasome enzymes, several kinases, ethylene signaling, transporters and proteins with DUF. Most of these molecular markers were validated by QTL and re-sequencing. The power of genomic selection will be enhanced by including phenomic information for understanding maize domestication and adaptation to changes in climate.

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P205

Identification of haploid immature embryos by their morphological difference in maize

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The doubled haploid (DH) breeding technology is being applied extensively and deeply in modern maize breeding and genetic research. Rapid and reliable identification of haploid is a key step to DH breeding technology but current systems were suitable for the selection of mature kernels. Here, we describe a new method for discrimination of haploid from diploid immature embryos based on their morphological difference. In our preliminary study, one inbred line and two hybrids were induced by three inducers with different oil content to confirm the morphological differences between the haploid and heterozygous diploid and find out the period of the biggest differences. The length, width and area of the heterozygous diploid immature embryos were significantly bigger than that of haploids, with the biggest differences during 16-18 days after pollination, which mainly showed the difference at the width and area. Compared with low oil inducer, the differences between the haploid and the heterozygous diploid immature embryos, which generated from high oil inducer, were more significant. Based on the difference of immature embryo shape of haploids and heterozygous kernels, haploid embryos could be discriminated effectively, and it worked better when based on both the width and area of immature embryos. In order to test the efficiency of the method for heterogeneous source materials, ten source germplasms, including inbred lines and the commercial hybrids, were pollinated by the same inducers above. We determined that the accuracy of haploid identification is influenced by the parent materials and the high oil inducer was superior to that of the low oil inducer. Under the premise to ensure the haploid number, 47.0%-75.8% of diploid could be rapidly eliminated before double treatment. In conclusion, this new method could extend the application and increase the efficiency of the double haploid technology in maize.

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P206

Inbreeding depression in wild maize populations (*Zea mays* ssp. *parviglumis*) subject to habitat degradation in southwest Mexico

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Crops were domesticated from wild taxa, in many cases, thousands of years ago. These wild progenitors can often still be found sympatric to their domesticated counterparts and can serve as a source of variation for the genetic improvement of modern varieties. However, the realization of this genetic potential depends critically on the conservation of wild populations. With little protection in place, concerns regarding habitat degradation and the overall decline of wild maize populations have prompted this study. Five populations of *Zea mays* ssp. *parviglumis* collected in Jalisco, Mexico were planted in a common garden using a randomized compete block design (RCBD) with five replicates. Eleven phenotypic traits correlated with plant fitness were measured. In addition, previously generated microsatellite genotypes of the five populations were evaluated to determine levels of neutral genetic diversity. Plants whose seed were sourced from larger populations had greater genetic diversity and possessed phenotypic traits associated with higher fitness, while plants sourced from smaller populations had traits characteristic of lower fitness. Plants from larger populations germinated more quickly, reached anthesis sooner, demonstrated a higher level of photosynthetic activity, and produced more biomass, suggesting a direct correlation between fitness of a population and genetic diversity. These results emphasize the importance of preserving large populations of *Zea mays* ssp. *parviglumis* to limit inbreeding depression and maintain the genetic diversity and adaptive potential of this germplasm.

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P207

Incorporation of functional information into genomic prediction models in maize

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Genomic prediction applied to plant breeding has achieved satisfactory accuracy for estimating the breeding value of individual plants. However, underlying models have typically utilized genomic marker data for capturing relatedness to individuals in a target breeding population, rather than depicting functional relationships between marked loci and phenotypic traits of interest. As a result, genomic prediction models have often lacked persistency in accuracy across population backgrounds.
This study aims to assess the benefit of annotation and expression information about marked loci to capture robust functional relationships between such loci and traits of interest. Here we develop and calibrate genomic prediction models on a diverse panel of 282 maize lines and test such models on breeding populations represented by 25 families in the Nested Association Mapping panel, each comprising up to 200 inbred lines. Genomic prediction models use as input genomic marker data, but also leverage predictions about selection intensity and/or gene expression levels of genes around marked loci. Selection intensity is based on conservation features such as GERP scores and gene expression levels are predicted based on motif detection by convolutional neural networks.
Our analyses assay the usefulness of diverse panels as reference for estimating breeding values in populations that are genetically distinct, but relevant to breeding applications. In such context, functional information may result in estimated effects of genomic loci that are more consistent across population backgrounds, hence reducing the importance of calibrating genomic prediction models on specific panels constrained to be genetically related to the target breeding population.

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P208

Influence of arbuscular mycorrhiza on stress resilience in a European maize diversity panel

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Climate change is predicted to increase extended drought periods in many regions and even in temperate climates. These pose threats to agricultural production and solutions to increase the stress resilience of crops are needed. Arbuscular mycorrhiza (AM) is a symbiosis of plant roots with AM fungi, which extend the reach of root systems through their extraradical hyphal network. AM improves uptake of mineral nutrients - especially phosphate (P) - and has also been described to increase drought stress resistance. Increased plant performance due to AM was reported to be subject to natural variation.
Here we examined the response to artificial AM-inoculation of the Dent parental founder lines of the maize EU NAM population (Bauer et al. 2013) under varying field conditions, including drought. The DH lines were grown for two seasons, with or without inoculation with the AM-fungus *Rhizophagus irregularis*. The three test sites were (1) a conventionally-cropped rain-fed field, (2) a field with drought stress induced by a mobile rainout shelter, and (3) a low-P field.
Roots of all lines showed high AM colonization, both in the *Rhizophagus*-treated plots and in control plots due to autochthonous AM fungi. Average root colonization across lines depended on the environment but did not change due to *Rhizophagus* inoculation. Year, environment and inoculation influenced root colonization of individual lines. Under drought stress, the average grain yield was higher in AM-inoculated lines than in control plots in year one, whereas no significant difference was observed in year two. In the rain-fed field no average yield improvements occurred in either year, whereas inoculation markedly improved average grain yield under low-P conditions. Individual lines profited differently from AM-inoculation in the different environments.
These results lay a basis for a further elucidation of the factors underlying host-symbiont functional compatibility and indicate that benefits from AM-inoculation depend on the environmental conditions.

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P209

Inheritance of short-season maize (*Zea mays* L.) senescence patterns

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Maize (*Zea mays* L.) exhibits two patterns of senescence in the late grain filling period, either rapid senescence of the photosynthetic tissues (die and dry) or delayed senescence (stay green). In short season environments such as Canada, these patterns of senescence are significant since green leaf tissue present at maturity can affect the moisture content of the grain and delay harvest. Although data on maize senescence patterns can be collected, late season confounding factors such as leaf diseases and environmental conditions can make accurate phenotyping difficult. The goal of this research is to develop a method for assigning senescence patterns early in the grain filling period to eliminate confounding factors, as well as to understand the inheritance of senescence patterns across many genotypes.
Preliminary observations from 4 genotypes over 2 years showed that response to sink removal in the early grain filling period correlated with senescence pattern. In the present study, 130 genotypes developed by a North Carolina Design II mating scheme were used to assess senescence pattern heritability. A fixed wing unmanned aerial vehicle (UAV) (Lancaster Rev5, PrecisionHawk) with multispectral RG-NIR and BG-NIR sensors were used to capture physiological changes caused by prematurely removing the primary sink. Sinks were terminated 3-weeks after silking in a single row of a 2-row plot. In addition, a chlorophyll meter (SPAD 502 Plus, Spectrum Technologies) was used to record chlorophyll concentrations at sink termination and plant maturity. This research is designed to test if senescence pattern is a heritable trait to aid breeders when making decisions on what germplasm to keep in breeding programs.

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P210

Insights into heterotic patterns and allelic diversity of U.S. dent maize from expired plant variety protection inbreds

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The privatization and consolidation of commercial maize breeding in the United States has resulted in a highly competitive industry. As a result, developers of elite maize inbreds pursue forms of intellectual property protection to secure their proprietary germplasm. One form of intellectual property protection is the Plant Variety Protection (PVP) system where novel inbred lines are protected for a period of 18-20 years. Upon expiration of the PVP certificate, the previously protected PVP inbred is publically released via the North Central Plant Introduction Station. Expired-PVP inbred lines can be an excellent source of elite germplasm to enhance or initiate a breeding program. However, this can be challenging due to the cryptic information on background and use of these lines, and frequent representation of novel nuances of heterotic families that have been optimized by multiple breeding cycles to novel germplasm pools within proprietary breeding programs. A panel of 328 expired-PVP inbred lines was genotyped over 899K SNPs derived from RNA-sequencing. A hierarchical cluster analysis grouped these expired-PVP lines based on genetic similarity into heterotic sub-groups. An ADMIXTURE analysis was performed to approximate the genomic presence of eight heterotic sub-groups. These analyses define the heterotic composition of the inbreds in the current set of publicly available expired-PVP germplasm. In conjunction with information provided in PVP certificates; the utilization of heterotic sub-groups was estimated for Pioneer Hi-Bred, Dekalb-Pfizer, and Holden's Foundation seed companies' elite germplasm from 1978-1998. To explore the effect of crossing between and among canonical heterotic patterns, 500 unique hybrids derived from expired-PVP inbreds were evaluated in a multi-environment yield trial experiment. This information can greatly expedite the process of utilizing this set of elite germplasm for the improvement of maize.

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P211

Reconstruction of Ancestral Genotypes in a Multiparental Parallel Selection Population

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Genetic diversity is fundamental to environmental adaptation and breeding. However, capitalizing on exotic germplasm, which harbors unique genetic variation, is challenging, because maladaptive phenotypes for some traits can skew the phenotypic effects for other traits being evaluated in their non-adapted, but targeted environment. Therefore, understanding the genetic response to selection for traits underlying adaptation is crucial for overcoming genetic barriers to crop improvement. We created a TROPICal Synthetic (TROPICS) population of maize from seven tropical inbred lines and performed parallel selection for early flowering time across a latitudinal transect (eight locations spanning from Puerto Rico to Wisconsin) for two generations. During each generation of selection and at each location, 384 individuals were genotyped from the extreme tails in flowering time and from the base population (~12,000 total samples) which have been genotyped using genotyping-by-sequencing (GBS). The optimum number of imputable markers was determined by assessing the accuracy of imputation as a function of different thresholds for genotype call rates. Using this maximized marker density, reconstruction of founder haplotype blocks for each of the 12,000 samples is being used to explore patterns of recombination and determine founder contributions in selected populations. Allele frequency differences between phenotypic extremes of the population were evaluated (extreme mapping), and several highly significant associations were detected across generations in the same location, but were not present across all environments. Populations from locations that exhibited the greatest phenotypic response to selection showed the greatest differences in allele frequency at markers with significant differentiation. Ultimately, integrating genome-wide imputation, reconstruction of ancestry blocks and tests for selection will provide new insight into the response to selection and the identification of potential barriers to environmental adaptation. These analyses are ongoing and the latest results from this study will be presented.

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P212

Maize resistance to parasitism : life-cycles synchronisation matters

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Plants can escape herbivores either by building up defenses such as plant secondary compounds, or by shortening their life-cycle so that they become mature before herbivore attacks. In France, *Lepidoptera* stem borers (pyralids) have a two-generations/year life-cycle. In spring, adult females lay eggs on young leaves. During summer, second generation caterpillars enter plant stems and dig galleries until ears. Completion of life-cycle occurs in non-harvested cobs during winter. Among tools for biological pest controls, sowing early maize varieties is recommended because maize stems are stronger when the second-generation larvae arise and better resist invasion.
To analyse how plant phenology shifts interfered with *Lepidoptera* stem borers life-cycle, we used the plant material coming from two independent Divergent Selection Experiments (DSEs) for flowering time in maize, that have been conducted in Plateau de Saclay for more than twenty generations. The two initial populations consisted in two seed lots, each from a single inbred line, F252 or MBS. At each generation, we selected and selfed early and late flowering plants. The resulting Early and Late evolved populations exhibited pronounced phenotypic divergence for flowering, while preserving original characteristics of the initial inbreds.
DSEs experimental designs from generation G20 and G21 were used to measure pyralids prevalence in Early and Late populations from either F252 or MBS genetic backgrounds. Altogether range of variation for flowering time was comprised between 1300 (mid-july, Early F252) and 1900 (mid-august, Late MBS) degree-days. Pyralids prevalence was more important in Early than in Late MBS populations. For F252, there was no differences between Early and Late populations, but average prevalence depended on the date of arrival of adults from the second generation (year effect). Hence, in our plant material, sensitivity to parasitism is more related to synchronization between insect and plant life cycles than to maturity of leaves.

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P213

Mapping loci that modify the efficacy of *Teosinte crossing barrier 1*

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Teosinte crossing barrier 1 (*Tcb1*) is a genetic cross-incompatibility factor that is responsible for blocking non-self-type pollen in silks. Originally found in teosintes, *Tcb1-s* (*strong allele*) has been introduced into modern maize varieties conferring resistance to *tcb1* pollen. Previous studies using a similar cross incompatibility system, *Gametophye factor 1* (*Ga1-s*) suggest that the cell wall modification enzyme *ZmPme3*, a pectin methylesterase, along with multiple modifying QTL loci contribute to the effectiveness of silks at resisting foreign pollen types. In *Tcb1*, little is known about the genetic modifiers and, more importantly, what the underlying biological mechanism is for this cross incompatibility. Cross-incompatibility systems like *Tcb1* and *Ga1* can be beneficial to breeders and farmers when only certain pollen types are desired on specialty maize crops. It was observed that nearly all the F1's of various inbreds, including B73, crossed by W22 *Tcb1-s* demonstrate strong incompatibility with *tcb1* pollen. One exception was Mo17, whose F1s had weaker resistance. In this study we used recombinant inbred lines (RILS) from the intermated B73-Mo17 (IBM) population crossed with homozygous W22 *Tcb1-s* plants to test the efficacy of the various F1s at blocking *tcb1* pollen. The F1s were tested by first challenging the *Tcb1-s* silks with *R1 C1 tcb1* pollen and the next day pollinated the same silks with *r1 c1 Tcb1-s* pollen. The resulting ears were scored for the percentage of colored kernels. Six quantitative trait loci (QTL) were detected on chromosomes 1, 3, 5, and 7 that explained 28.9% of the phenotypic variability. Most modifying QTL loci showed simple additivity effects and epistatic interactions between loci. Further exploration into these genomic regions and the underlying candidate genes is underway, these results could shed light on the genetic and physiological mechanisms controlling *Tcb1*.

Funding acknowledgement: National Science Foundation (NSF), SDSU Experimental Station

P214

Mapping QTLs for Kernel Row Number and Fasciated Ear by SNP-Based Bulk Segregant Analysis in Maize

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In maize, the number of kernel rows (KRN) of the ear is one of the most important grain yield components. Both QTLs and Mendelian mutations (such as abnormally shaped - Fasciated - ear mutants) have been discovered for this trait and utilized to gain information on the molecular genetic control of ear development. In this study, two Italian maize inbred lines were identified to show extreme phenotypes in terms of ear fasciation and low KRN, respectively and utilized to develop three recombinant inbred line (RIL) populations. Two of the populations (A and B) had the fasciated ear type inbred line as parent, while the third population (C) was generated by crossing the elite line B73 with the low KRN line. The three populations were thoroughly phenotyped for ear morphology and KRN in F5 and F6 generations and showed an overall continuous type of variation for ear traits. We next attempted to map QTLs for fasciated ear and KRN using bulk segregant analysis (BSA) based on a high-density maize SNP array (15k Illumina Infinium) in two successive years. Bulks included 15 plants (extremely fasciated ear plants or wild-type ear plants for populations A and B, and plants with highest or lowest KRN for population C). Preliminary results showed the presence of major QTLs segregating and affecting both ear fasciation and KRN.

P215

MAZE - Accessing arbuscular mycorrhiza-mediated drought stress resistance in a maize diversity panel

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Drought is one of the major constraints limiting productivity of crops, such as maize, worldwide. Methods and tools to limit negative effects of drought on yield are highly desired. Arbuscular mycorrhiza (AM) is a mutualistic symbiosis between more than 80% of land plants and fungi of the phylum Glomeromycota. The fungi provide plants with mineral nutrients in exchange for carbohydrates and lipids. AM associations with plants have been shown to enhance maize performance under drought stress conditions. Previous studies showed that the positive effect of AM symbiosis on plant performance depends on the maize genotype, suggesting that AM responsiveness is subject to genetic variation. To identify genotype specific AM-responsiveness we aim at investigating drought stress resistance of 24 European maize Dent inbred lines in association with the AM fungus Rhizophagus irregularis. In pilot experiments, we show increased root and shoot fresh weight of maize plants associated with R. irregularis. Currently, we are establishing conditions to perform a phenotypic screen to characterize genotype-dependent performance differences in response to AM fungi under well-watered and drought stress conditions.

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P216

Natural variation for starch composition and processing characteristics of maize

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Nixtamalization is an alkaline cooking process used in the production of corn chips and tortilla products. The cooked kernels, referred to as nixtamal, need to have uniform moisture profiles within batches to ensure proper processing and quality control of the final product. However, the compositional factors affecting water uptake during nixtamalization are not well understood. Amylose and amylopectin content are hypothesized to significantly affect nixtamal moisture, as the two starches have well characterized differences in moisture uptake and retention. To test this hypothesis, we selected 100 spectrally and genetically diverse lines for compositional analysis. Amylose and amylopectin ratios were analyzed with high performance liquid chromatography (HPLC). Amylose chain lengths were also measured using an iodine affinity assay. Nixtamal moisture content was measured using a small scale nixtamalization assay. Significant variation in nixtamal moisture (35-55%) was observed, with amylose in the starch ranging from 69 to 86%, and the remainder of the starch being amylopectin. These traits are being incorporated with other compositional traits including total starch, protein, fiber, oil, and sugar in a machine learning framework to build a model to predict nixtamal moisture content.

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P217

Navigating the maize of short-season ancestry

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Preliminary statistical analysis on early, intermediate, and late maize germplasm lines suggest that the early germplasm pool have a broader group of ancestral lines than what was originally proposed. A series of NC Design II yield trials were conducted in 2016 as part of the Genomes to Fields (G2F) project. Lines from recently expired PVP certificates (off-PVP lines) or second generation lines of the off-PVP lines were chosen based on diversity of originator (company and geographic) and on usage in commercial hybrids. The lines were also stratified to generate a set of hybrids for testing in the Early G2F environments, the Intermediate and the Late G2F testing environments. The early group is believed to have less genetic diversity due to a genetic bottleneck from selection for earliness. However, the DII Early set exhibited the greatest genetic variation as a percentage of the total variation for grain yield (23% vs. 15% vs. 5%), plant height (12%, vs. 6% vs. 5%), and days to silking (9%, vs. 3%, vs. 1%). The DII Early set exhibited significant additive genetic variation in both the males and females for grain yield, however, the DII Intermediate and DII Late sets did not exhibit any significant genetic variation on the female side for grain yield. This has led to the hypothesis, the modern early season germplasm base has founder lines that are not part of the founders of the corn belt dent germplasm base, which will be examined in this study. A sample of short season germplasm represented by inbred lines from AAFC, NDSU, early flowering off-PVP lines, and CG lines derived from short season commercial hybrids will be used to determine the relationship of the proposed 7 founder lines with this germplasm pool and what this germplasm pools most likely founders lines are.

Funding acknowledgement: NSERC, OMAFRA

P218

Omics-based prediction of hybrid grain yield in maize

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Modern plant breeding uses hybrid breeding to achieve higher yields by exploiting heterosis, the increased vigor of hybrids over their parental inbred lines, as well as taking advantage of uniform F1 populations.
Large numbers of parental inbred lines can be generated through doubled-haploid technology, but it is not feasible to evaluate all their combinations in field trials. Genomic prediction supports the selection of inbred lines and their combinations which are most promising to result in high hybrid grain yield and thereby increases the efficiency of the selection process.
In this study we analyzed the relation of parental differences in SNP, messenger RNA expression and small RNA expression profiles with hybrid grain yield. While larger differences in SNP and messenger RNA expression of parental inbred lines are related to higher hybrid grain yield, the opposing trend was observed for small RNA expression.
We developed a prediction approach relying on positively and/or negatively associated differences of the analyzed omics datasets with hybrid grain yield. The prediction accuracies were compared for different prediction approaches as well as test-cross scenarios for all three omics types. The comparison resulted in the conclusion that different omics types and prediction approach variants should be favored depending on the test-cross scenario to provide the optimal prediction accuracy.

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P219

Phenotypic and genetic dissection of cold tolerance in maize using controlled environments and in-field evaluations

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Earlier planting is an important factor that positively contributes to increased maize yields. Identifying varieties that combine improved germination, emergence, and vigor at early growth under suboptimal temperatures is an important breeding objective. Our goal is to determine the genetic architecture of maize cold sensitivity in controlled environments as it relates to in-field performance. A set of 33 inbred lines were selected for their cold tolerance variability and were evaluated in replicated field trials in 2016 and 2017. Replications were planted on March 22, March 29, and April 5 both years, 46 - 26 days before optimal planting, May 1, which generated cold temperature treatments. Seedlings were screened for emergence, the percentage of plants whose coleoptiles penetrated the soil surface from the total planted, and seedling vigor, a rank scale which combined emergence, emergence rate determined by growing degree units (GDUs) to 50% emergence and growth rate determined as GDUs from leaf stages V1 to V5. Germination ranged from 0 to 93%, while 5 genotypes ranked highly cold vigorous (1 - 2) and 8 highly cold susceptible (9-10). Inbred lines B97 and P39 were among the most resistant genotypes, whereas B73 showed relative susceptibility to suboptimal temperatures. Given the diverse performance of these three lines, we evaluated the recombinant inbred line (RIL) of the Nested Association Mapping populations B73 X B97 and B73 X P39 in two controlled environments with contrasting temperature treatments, 14°/12°C (cold) or 26°/24°C (control) 12 hour day and night cycle. We deployed a high-throughput imaging platform, the Maize Architecture Analysis Station (M.A.P.S), to screen emergence and seedling growth for 200 RILs from each population replicated twice per controlled treatment. The information collected will further our understanding about the genetic architecture of this economically important trait and facilitate the comparison of phenotypic response of controlled environment evaluations with field conditions.

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P220

Phenotypic and genomic indicators of breeding program sustainability: application to a north European grain maize program

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Successful plant breeding programs rely on balanced efforts between the development of competitive cultivars in the short term and the maintenance/improvement of genetic diversity to guarantee longer term progress in the genetic pool. Response to selection per breeding cycle is determined by selection intensity, selection accuracy as well as the additive genetic variance of the trait. Hence, characterization of additive genetic variance and its determinants in breeding populations is important for evaluating the progress expected from selection.
We propose indicators based on temporal phenotypic and genotypic data to analyze realized genetic gain and evaluate its sustainability. Indicators were applied to a private (RAGT2n) early maize grain program implying a Dent-Flint heterotic pattern.
The joint analysis of genetic gain and additive genetic variance over one decade of selection revealed a significant positive genetic gain for grain yield in both Dent and Flint populations, along with a tendency towards a decrease of the additive genetic variation. Recent genetic diversity introduction in the Flint population contained this reduction and resulted in a higher additive genetic variance compared to the Dent population.
High-throughput SNP genotyping highlighted a slight trend towards a decrease in diversity in Dents and a more stable diversity in Flints. It permitted to identify regions of the genome with a particularly low diversity, mainly localized in pericentromeric regions, which might gain to be enriched by new introductions of external genetic resources.
Since temporal series of phenotypic and genotypic data are available in most public or private breeding programs, the presented approach can easily be implemented in the context of Genomic Selection.

Funding acknowledgement: RAGT2n, CIFRE-ANRT, INRA

P221

Plant phenotyping reveals genetic and physiological factors of plant performance

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Whole plant phenotyping integrated with genotyping and molecular profiling is used to uncover determining factors and mechanisms of plant (growth) performance. It relies on IPK facilities for automated cultivation, transport, and imaging of plants in climate controlled phytotron/glasshouse cabins equipped with diverse camera and illumination systems and a broad range of environmental sensors. Beyond GWAS-based detection of QTL for final biomass, water consumption, and water use efficiency, repeated non-invasive size monitoring of 261 maize dent lines revealed the complex genetics of growth dynamics: 12 main effect QTL and 6 pairs of epistatic interactions displayed markedly different temporal patterns of activity. Some also affected relative growth rates and 4 additional growth dynamics QTL were detected using nonparametric functional mapping and multivariate mapping approaches. Thus, integrated time-resolved analyses are required addressing further physiological (e.g. PS II efficiency) and architectural features found to vary strongly among IPK Genbank accessions.

P222

Plant Transformation Services

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University of Missouri (MU) Plant Transformation Core Facility was established in 2000. The key mission of the Facility is to enhance both basic and applied plant biology research by providing plant transformation services and advancing transgenic technologies. Since 2000, the Facility has been providing state-of-the-art plant transformation services. The services are on fees for cost recovery only, not for profit. The facility staff is dedicated to providing various types of transformation services with a focus on maize (*Zea mays*), soybean (*Glycine max*), switchgrass (*Panicum virgatum*), and sorghum (*Sorghum bicolor*). The service categories include both standard and customized transformation. Transformation systems for all crops utilize Agrobacterium-mediated approaches and somatic embryogenesis processes except for soybean. The *Agrobacterium*-mediated cot-node transformation system coupled with organogenesis regime is employed for soybean transformation. Research activities are geared towards developing high-throughput transformation systems, effective small RNA-mediated gene silencing, gene stacking through coordinated transgene expression, and precise genome modifications to meet the needs of crop improvement and genome discoveries. More details on the facility operations and experiences as well as its impact on research collaborations and funding opportunities will be discussed wherever appropriate during the presentation. Readers should find out more about MU Plant Transformation Core Facility at http://plantsci.missouri.edu/muptcf/, email to Zhanyuan J Zhang at zhangzh@missouri.edu, or call facility office at 573-882-6922 for specific service questions.

P223

Population structure and genetic diversity of different maize genotypes

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Knowledge of genetic diversity and population structure among inbred lines provides a better understanding of the genetic relationship between genotypes. A set of 40 maize inbreds belonging to five different kernal types (white, yelow and orange kernel, sweetcorn and popcorn) from Gene Bank of Maize Research Institute „Zemun Polje“ was subjected to molecular marker analysis with the aim of revealing genetic diversity and population structure. Preliminary research was done using 20 informative SSR markers. Total number of detected alleles was 104 with an average of 4.3. PIC value for all tested markers was greater than 0.6. The greatest Ho was detected for sweetcorn while the lowest for white kernel maize group. Number of private alleles was the highest for orange kernel maize group and the greatest number per genotype (4) was found for yelow kernel maize genotypes. Better congruence with pedigree information was revealed using PCA than cluster analysis. Variability among individuals was greater than inter-population variability which is not unexpected conidering the fact that lot of inbreds inside one group are of very different origin. Selected genotypes will be further investigated with a greater number of markers in order to better describe Gene Bank genotypes which will be used in future breeding programs to better explore the genetic variability within and between groups to generate new lines and hybrids.

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P224

Post breeding

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The EU regulates the marketing of plant reproductive material of plant species, ensuring that EU criteria for health and quality are met. EU legislation is based on: registration of varieties or material and certification or inspection of lots of seed and plant propagating material before marketing. The common catalogues of varieties of agricultural plant and vegetable species list the varieties which can be marketed in the EU.
In Italy the procedure to list a new maize variety contemplates two years of field trial, coordinated by CREA-DC. The variety will be list if it meets standards on: Distinctness, Uniformity, Stability (DUS test) and Value for cultivation and use.
A key point in this work is to establish the distinctness of new plant material from all varieties of common knowledge. Candidate varieties will be compared first on the paper (official description) and second side-by-side in the field with similar other varieties already knowledge present in our reference collection (RC). In the RC, indeed, should be present all varieties listed or protected (CPVO-UPOV); any other variety in common knowledge. The establishment and the maintenance of the RC for comparative testing and evaluation in growing trials lead to considerable time and cost expenditures, and large reference collections may be a major challenge for Examination offices (EOs).
Besides some characteristics, observed for the description of the new variety during the DUS test, have strong environmental influences as a result the valuation of the appropriate state of expression is not as much reliable within the years and between the EOs.
In this time the network of entrusted Examination Offices is working to develop innovative solutions to face difficulties of ever increasing trial size and related costs, as centralization of DUS of certain species, R&D projects to build harmonize databases including e.g. phenotypical data (ring tests amongst entrusted EOs as prerequisite) and molecular profiles and pictures.

P225

QTL for photosynthetic and yield performance in IBM population under two different heat scenarios during flowering time

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It was demonstrated that extreme heat as a stressor could have more critical role for maize production than drought in temperate areas. Accumulation of extreme temperatures (>30°C) is associated with increased vapor pressure deficit (VPD) as a function of temperature and relative humidity used recently in maize genetic studies. The objectives of this three-year study were 1) to compare photosynthetic performance during flowering with grain yield, and 2) to analyze quantitative trait loci (QTL) for the performance in testcrosses of IBM population under two different heat scenarios. PIABS - performance index for energy conservation from photons absorbed by PSII to the reduction of intersystem electron acceptors was used as a parameter for the photosynthetic performance. Two distinct geographic regions of Croatia (Osijek) and Turkey (Ayvalik) differed considerably in VPD during flowering time (in average 2.5±0.3 and 3.4±0.3 kPa, respectively). The correlations between PIABS and yield were notably stronger in environments with higher VPD (Turkey) reaching r=0.59. Inclusive composite interval mapping (ICIM) revealed a larger number of significant, but mostly environmentally dependent QTLs for PIABS and grain yield. Some of them were associated to tolerance to heat stress because they were specific to heat scenarios. Our complete quantitative genetic analysis for series of photosynthetic and agronomic traits will be used for assessing the photosynthetic performance of maize genotypes as a function of grain yield in heat stress environments.

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P226

QTL mapping and genomic predictions for silage quality traits in a multiparental hybrid design

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Maize (Zea mays L.) is commonly used as silage for cattle feeding or for biogas production in Northern Europe. Improving whole plant biomass degradation is therefore a major breeding objective. Hybrid varieties selected for silage generally result from crosses between Dent and Flint heterotic groups. It is therefore of key interest to identify loci (QTL) involved in the General (GCA, parental values) and Specific Combining Ability (SCA, cross specific value) components of hybrid value and find out in each group favorable alleles that can be combined in hybrids. We implemented an original factorial design of 951 Flint x Dent hybrids obtained by crossing inbred lines issued from two multiparental connected designs, one specific of each group. Inbred lines were genotyped for 20K SNPs and hybrids were phenotyped in eight environments for silage production and quality traits (measured by near infrared spectroscopy). For each quality trait, we (i) estimated GCA and SCA variance components, (ii) performed QTL detection using new approaches adapted to our hybrid design and (iii) genomic predictions of GCA and SCA components. We found a predominance of GCA over SCA in hybrid genetic variance. In total 230 QTLs were detected with only two showing significant SCA effects at the whole genome level and more than 80% of GCA QTL were specific of one heterotic group. Each QTL explained less than 10% of the within-population phenotypic variance and 96% of them co-localized with at least one QTL of another trait suggesting pleiotropic effects. Good GCA predictive abilities were obtained with both QTL and genomic models but genomic models outperformed QTL ones. These results illustrate the complex genetic architecture of silage quality traits and are consistent with the divergence between heterotic groups. This work opens new prospects for improving maize hybrid performances for both biomass productivity and degradability.

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P227

QTL mapping for fusarium seedling rot resistance in the recombinant inbred crosses derived from MAGIC maize population

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Fungal infection by *Fusarium verticillioides* is cause of substantial reductions in maize yield and grain quality worldwide. Developing natural resistance in maize genotypes is an effective way to achieve sustainable control of *F. verticillioides* in the field, and breeding for resistance may be accelerated by identifying the quantitative trait loci (QTL) responsible for disease resistance. The multi-parent advance generation intercross (MAGIC) maize population, built by the intercross of eight diverse inbred lines, was used to identify resistance QTL on a set of highly diverse recombinant inbred lines (RILs). Phenotyping for *F. verticillioides* seedling rot (FSR) resistance was conducted with a rolled towel assay (RTA) allowing a fast screening to identify resistant RILs. We identified three major FSR QTL that were further dissected using transcriptomic and sequencing data to reveal candidate genes. As maize is cultivated mostly as hybrids, the study of *F. verticillioides* resistance in hybrid background may significantly contribute to the production of maize hybrids having higher field resistance. We developed a novel maize population crossing the MAGIC RILs to produce recombinant inbred crosses (RIXs). RIXs were produced by crossing RILs in a chain design over two seasons in Italy. Their genomic diversity was derived from the haplotype composition of RILs in each crossing pair, thus not requiring further genotyping. The RTA screening for FSR was performed so far on 250 RIXs, reporting broad variation for the trait. These results will be used to study the heterotic contribution to FSR resistance in maize.

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P228

QTLs affecting sweet corn carbohydrate content and eating quality in *sugary1*

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There are three main endosperm genotypes for commercial sweet corn varieties: sugary *(su1)*, sugary enhancer *(su1se1)* , and supersweet *(sh2)* . The "sugary enhancer" genotype is a double mutant *su1/su1, se1/se1* and widely grown in fresh market production. Sugary enhancer quality has been attributed to an excellent texture, good flavor, elevated sweetness, and a thin pericarp. However, sugary enhancer genotype trials show inconsistencies in sugar and starch accumulation indicating other loci also contribute to sugary enhancer quality. The objective of this research is to identify quantitative trait loci (QTLs) of modifier loci of sugary enhancer quality in a *su1* background in sweet corn, using F_5 recombinant inbred lines (RILs) derived by single seed descent (SSD). Two populations were developed from biparental crosses of divergent sweetness, one heterozygous for *se1* and one homozygous. A linkage map of 20,000 SNPs was developed using a genotyping by sequencing (GBS) method. With this, novel QTL on chromosome 5 and 6 were found to influence sucrose, total sugar and starch content in Population 1. A QTL matching the *se1* region was found to be most significant in Population 2. A new predictive platform using Near Infrared Spectroscopy (NIRS) was developed to measure starch, phytoglycogen, total sugar, sucrose, fructose, and glucose at the fresh eating stage and at the dry harvest stage. This was validated with 10% random subsample using enzymatic lab assays and has a high throughput predictive ability with a high R^2.

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P229

Relationships between resistances to Fusarium and Aspergillus ear rots and mycotoxins contamination in maize kernels

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Ear rots caused by different Fusarium and Aspergillus species are one of the most dangerous food and feed safety challenges in maize production. The majority of the inbreds and hybrids are susceptible. The aim of this research was to identify inbred corn lines resistant to pathogenic and toxigenic species - Fusarium graminearum Schwabe, Fusarium verticillioides (Sacc.) Nirenberg and Aspergillus flavus Link and fumonisin and aflatoxin accumulation. In field trials, a select group of 40 inbred lines was inoculated into the silk channel with fungal spore suspension. After harvest, ears were rated for rot and evaluated for levels of aflatoxin or fumonisin contamination. Only four inbred lines showed high level of resistance to all three investigated species, and had low levels of both mycotoxins contamination. The highest number (65%) of investigated genotypes showed moderate resistance to all three tested pathogenic species. A highly significant correlation between the resistance to F. verticillioides and Aspergillus flavus was established. However, the correlation between the resistance to Fusarium graminearum and the other two species was positive, but not statistically significant. Area of the ear rotted by F. verticillioides and A. flavus was significantly correlated to toxin production for both fumonisin ($r = 0.69$) and aflatoxin ($r = 0.51$), indicating that inbreds aflatoxin resistance may also be good sources of fumonisin resistance.

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P230

Romania's 3,000 inbred lines collection as a reservoir of genetic diversity and the use of its cytolines in linking phenotype to genotype

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A collection of more than 3,000 maize inbred lines is being preserved in Romania's six stock centers, the Agricultural R&D Station, Turda, being the most important one. These lines are a "hidden treasure" worth investigating. We previously genotyped a subset of 90 inbred lines from this collection (Suteu et al., 2013, PLoS One) uncovering its allelic richness and therefore its potential in breeding programs worldwide. We are currently analyzing 250 more inbred lines and we are planning to genotype-by-sequence 1,200 more this year, in an effort to unlock the full potential of the Romanian germplasm in terms of predictive breeding. Concurrently with harnessing the power of heterosis to create superior hybrids, (based on the heterotic pools defined by genotyping) local breeders have also created cytolines by transferring the nucleus of valuable inbred lines to other cytoplasms through repeated backcrosses. Such cytolines show improved phenotypical traits. Using RNA-seq, we have defined a core set of genes that are differentially regulated in the cytoplasm-donor lines compared to their nucleus donor line in relation to three traits of interest (yield, taller plants and increased resistance to Fusarium). We argue that the genes we identified are responsible for the cytolines' improved phenotypes.

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P231

Root phenotypic and transcriptomic variation in the European maize landrace Petkuser in response to cold

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Most commercial maize cultivars in temperate Europe are not sown before May due to their cold susceptibility. Increasing their cold tolerance can prevent yield losses, which occur due to late cold stress events during spring. Furthermore, cold tolerant cultivars have greater yield potential due to an earlier sowing date. European landraces have a great yet untapped potential in improving cold tolerance, as they harbor high genetic diversity for cold tolerance, which can be made available to breeders. The response of plant roots to environmental stresses like cold is not well understood up to present. However, roots have essential functions for plant growth such as anchorage and the uptake of water and nutrients. We aim to assess the phenotypic diversity present in European landraces with respect to cold tolerance.
Therefore we screened a doubled-haploid population generated from the landrace Petkuser for cold tolerance during early root development using high-throughput controlled platforms and automated analyses of root traits. Significant genotypic variation for cold tolerance was found within the population with growth rates ranging from 16% to 72% at cold conditions compared to control conditions. To assess the influence of gene expression differences on cold tolerance we selected a set of 20 extreme genotypes for transcriptome sequencing, which is currently in progress.
Identification of candidate genes for cold tolerance and the association of gene expression with phenotypic traits related to cold tolerance in roots is a major focus of our experiments and will help to further understand the genetic mechanisms controlling cold tolerance in maize. These results will also help to improve breeding of cold tolerant cultivars.

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P232

ThaliaDB, a tool for data management and genetic diversity data exploration

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Diversity and association genetics studies lead to manipulate a large number of individual, lines, clones and/or populations. Moreover, emergence of high-throughput technologies for both genotyping and phenotyping generates a large amount of data. These data need to be stored and managed in order to make requests and to organize datasets to be able to perform genetic diversity data exploration and association genetics analysis. The new version of ThaliaDB, V3.2, is developed for scientists to facilitate their data management and analysis. The database holds genetic resources data (germplasm/accessions), seed lots, samples, markers and genotyping and phenotyping datasets (fields environments, multiple traits and conditions). It is well adapted for data, useful to apply GWAS or genomic selection methods. It can manage high-throughput results coming from different projects and experiments and propose several views and options to explore these data and to give access to them for reuse. This Web tool offers to users a Select (Data view) mode and an Admin (Data administration and loading) mode. Data confidentiality is maintained using user accounts and specific levels of rights can be set on data. It enables data extraction in CSV format. A version exists today in our lab with maize data produced from projects of A. Charcosset's GQM S team and theirs partners. Perspectives are to use it for tomato, wheat and poplar data. The software is currently in improvement with funding of Amaizing, Investment for the future, project. It is developed in Python under Framework Django, running under PostGreSQL and MongoDb databases management systems. Contact: delphine.steinbach@inra.fr for more information and collaboration.

Funding acknowledgement: French National Agency (ANR) and French National Institute for Agnonomical Research (INRA)

P233

The dynamics of adaptive response under strong selection regime in small populations

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Two independent Divergent Selection Experiments (DSEs) for flowering time in maize have been conducted under agronomical conditions in Plateau de Saclay for more than twenty generations. The two initial populations consisted in two seed lots, each from a single inbred line. At each generation, we selected and selfed early and late flowering genotypes. By selecting from such a narrow genetic basis, we expect to have enriched populations for (epi)genetic differences controlling flowering time, while preserving the original characteristics of the initial inbreds. Using genetic markers and transcriptomic data, we identified a number of (epi)genetic differences. In order to address questions related to the role of new mutations versus standing variation in the response to selection, and to the rate and limits of adaptation, we have implemented a revised version of the animal model that explicitly accounts for new mutations. In this model, the observed response to selection is treated as a quantitative trait, driven either by shifts in average phenotype or plastic changes. From the dynamics of the selection response, we quantified the input of new mutations over generations. In addition, we implemented a population genetic model that describes the fate of a new mutation, in this high selection-high drift design. We discuss how, in these conditions, drift can accelerate fixation of adaptive mutations.

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P234

The effects of artificial selection on stability and GxE in the Iowa stiff stalk synthetic maize population

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A plant's expressed phenotype is a function of the genotype, the environment and the differential response of genotypes to variable environments, known as the genotype-by-environment (GxE) interaction. While GxE affects plant performance, little is known about how artificial selection for high productivity has altered this adaptation response of genotypes to environmental variations. The focus of this project is to assess how selection has affected various agronomic traits, how GxE responses have been altered through selection and to determine biological controls of GxE due to this selection process. In 2016 and 2017, inbreds ranging from highly selected (recently expired plant variety protection) to founder lines from the Iowa Stiff Stalk Synthetic (BSSS) maize population were crossed to the tester 3IIH6 and evaluated in replicated trials across 32 locations as part of Genomes to Fields GxE Project. Agronomic and productivity traits were measured for the set of hybrids. GxE explained 13.8% of the phenotypic variability of grain yield. To evaluate the performance of unselected and selected lines relative to each other across environments, a Finlay-Wilkinson regression and stability analysis were used. Yield slope and mean square error (MSE) from the regressions demonstrate that selection for yield in maize lines resulted in a decrease in slope range from 0.6-1.3 (environmental mean/hybrid) in the selected set to 0.2-1.5 in the unselected group. There was also a significant reduction in the range of MSE from 1.6-4.0 in the selected set compared to 1.1-9.9 for the founder set. Genotype responses of slope near 1 and reduction in MSE suggest that the highly selected lines are more stable in performance across environments compared to lines that have undergone less selection. This work will enable future phenotype-genotype associations of phenotypes and genotypes to enhance our understanding of GxE and breeding approaches.

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P235

The effects of host and environment on the maize microbiome

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Every maize plant has billions of microscopic organisms living in, on, and around it. These microbial communities—collectively called a "microbiome"—have great potential to influence plant growth, but the extent of their influence and the degree to which the host plant shapes them is unknown. We address the question of how much influence the host and the environment have on microbiomes by looking at bacteria in the maize rhizosphere (roots) and phyllosphere (leaves). These two communities show very different makeups: the rhizosphere is highly complex, while the phyllsophere is dominated by <20 bacterial taxa. Environment is the largest driver of the rhizosphere community; host genetics has little direct effect but does show strong gene-by-environment interaction. Broad- and narrow-sense heritability analyses in both communities show that a subset of microbes are moderately affected by host genetics (heritability of 0.3-0.6), while the majority show little effect of the maize genotype. We also identify several metabolic pathways in the phyllosphere that may be shaped by host genetics. Taken together, these results indicate that the maize host exerts only partial control over the makeup of its rhizosphere and phyllosphere communities, and in many cases the environment and/or stochastic chance play a larger role. In addition, the metabolic capacity of these communities may be more important than their taxonomic identity. More work needs to be done to determine to what extent these communities affect their host plant, and to determine if and how manipulating them can benefit agriculture.

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P236

The phenotypic characterization of the BALANCE maize panel reveals high potential to discover genetic determinants involved in drought response

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Relevant phenotyping plays a major role to understand the genetics of quantitative traits involved in the adaptive response to drought. The high phenotypic diversity of the BALANCE maize panel (derived from a MAGIC population) makes it a suitable material for the analysis of such a trait and for high throughput phenotyping efforts. To target the morpho-physiological traits that affect yield in drought condition, the BALANCE panel has been phenotyped in a network of field experiments in contrasting environments. Twenty-seven field experiments (combinations location x year x water regime) were performed across France, Hungary, Romania and Chile. Classical agronomical traits such as yield and its components, flowering time, plant height were collected in the field trials. Innovative phenotyping, such as spectral reflectance measurements using multispectral sensors mounted on a UAV, were also measured in a few trials: this gives access to vegetation indices, growth rate and stay green through the plant cycle. Root architecture in relationship with drought condition is investigated in a greenhouse platform through the current MIRGA project (ANR Funding). Environmental conditions are taken into account in all experiments in order to classify trials into well-defined stress scenarios. Soil water status is characterized to model water balances in the field. Adapted experimental designs and statistical approaches are used to improve the accuracy of phenotypic values. The heritability for each trait is calculated. This accurate and relevant phenotyping of the BALANCE panel developed by BIOGEMMA coupled with its deep molecular characterization makes it an attractive resource to better understand mechanisms underlying adaptive response to drought stress and ease the identification of relevant candidate genes.

P237

The ProSpect of traditional and molecular breeding.

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In modern breeding, many different selection strategies are at the disposal of the breeder. Which one to use depends on three key factors: i) genetic complexity of the trait(s) that need to be improved, ii) number of traits to be improved simultaneously, and, iii) available resources (financial, logistics, etc.). Currently, the decision often relies on experience, tradition and intuition, and draft cost calculations for candidate scenarios. However, when multiple and more complex traits are being set as targets for a new breeding program, choosing the optimal selection strategy may become extremely challenging. In such circumstances, it is valuable to be able to integrate knowledge and experience with tools to simulate and evaluate breeding scenarios in silico. This allows a breeder to systematically consider many of the variables that impact a breeding program. The breeding platform ProSpect developed by KeyGene provides exactly such a system. The platform provides an open strategic decision support system to explore costs and benefits of a wide range of breeding scenarios. Most notably, the system enables testing the most advantageous applications of three major selection strategies, i.e. phenotype selection (PS), Marker Assisted Selection (MAS) and Genomic Selection (GS), and combinations of those in the context of complex breeding programs. ProSpect enables users to find answers to their breeding questions not only by running predefined simulations, but also by further developing the platform according to their own specific needs. Results from different selection strategy scenarios will be shown and compared.

P238

The Zea French Biological Resource Centre: conservation and utilization of maize genetic resources in France

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Maize genetic resources are organized in France through the French maize genetic resources network. It includes public (INRA) and private actors (ProMaïs association). Maize Genetic resources include 1600 open pollinated populations and 4600 inbred lines. Their management is run by the Zea BRC (Biological Resource Center) shared between the UMR AGAP (populations) and the maize Experimental Unit of Saint Martin de Hinx (inbred lines). At national level, the Zea BRC is included in the French network RARe (Infrastructure Ressources Agronomiques pour la Recherche) and in the Plant network ARCAD. The basic activities of the Zea BRC are conservation, distribution and multiplication of populations and inbred lines. Genetic resources are distributed following international rules either with the International Treaty on Plant Genetic Resources for Food and Agriculture, the Nagoya Protocol or private rules (depending on the accession status). Information on genetic resources is available in the national database (Siregal) and a national portal (Florilège). Information on the traditional cultivation and use practices of populations can also be found on the Promaïs website.

A large fraction of these genetic resources have been characterized for their genotypic diversity and phenotypic variation by the CRB and partner research labs. Genotyping of populations and first cycle inbred lines revealed new features regarding the introduction and spread of maize in Europe, as well as local geographical trends (Brandenbourg et al., 2017, Nicolas et al., Diaw et al., this meeting). Broad or heterotic group specific panels of inbred lines have been defined within CornFed, DROPS and Amaizing projects to conduct Genome Wide association mapping revealing key loci for flowering time, heat and drought tolerance (Millet et al., 2016, Bouchet et al., 2017, Gouesnard et al., Blein-Nicolas et al., this meeting). These valuable resources allowed the development of multi-parental populations and introgression libraries to further dissect the genetic mechanisms of adaptation traits.

Discussions have been started at European level, to build a European Zea network to reinforce the means in conservation ant utilization of European maize genetic resources. The network would also permit a better sharing of taskforces and means in Europe and avoid gaps and losses in temperate genetic resources in Europe.

P239

Three chromosomal segments have a strong effect on photosynthesis and ability of maize plants to transition to autotrophy under chilling conditions

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In maize, transition to autotrophic growth is hampered by cold temperatures. Plants from susceptible lines can die after seed reserves have been exhausted if temperatures remain below 13°C. We used genome-wide association mapping or linkage analysis to identify quantitative trait loci related to the ability of plants to transition to autotrophy under chilling conditions.
We analysed a highly diverse panel of 293 dent lines. This panel was genotyped with a 50k SNP array and by sequencing, providing approximately 500,000 useful markers for genome-wide approaches. The lines were crossed to a flint tester and phenotyped as hybrids in cold greenhouses. We also analysed two doubled-haploid populations derived from crosses between European dent inbred lines. The two populations of about 100 individuals each were genotyped with a 50k SNP array and approximately 700 markers from framework maps were retained for QTL mapping. The inbred lines were phenotyped in cold greenhouses. Each panel or population was evaluated for several traits including vigour rating after 4 weeks of cold treatment. For each panel or population, a major QTL for vigour rating was highlighted.
Near-isogenic lines for each of these 3 QTL were constructed by marker-assisted backcrossing in cold-tolerant or cold-sensitive recipient line followed by selfing. Pairs of QTL-NILs were phenotyped in a cold growth chamber. All lines carrying the sensitive allele showed a strong reduction in vigour rating. A significant depression of chlorophyll content and quantum yield efficiency of photosystem II was observed for the lines with a cold-tolerant background and plant mortality occurred for the lines with a cold-sensitive background.
Further studies in field conditions will help breeders to decide how much attention they should pay to such alleles in their breeding programs.

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P240

Towards cloning of a major chilling tolerance QTL in maize

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Chilling tolerance is an important prerequisite for cultivation of high-yielding energy maize in central and northern Europe. QTLs for chilling tolerance were identified in a double-haploid population constructed by a cross of a chilling-sensitive and a chilling-tolerant line. One of the major QTLs explaining 34 % of the phenotypic variation was mapped on chromosome 4. The QTL was validated by field tests of Near Isogenic Lines (NILs) carrying small introgressions of the tolerant line in the genetic background of the sensitive line. Different types of markers were developed to narrow down the QTL to an interval accessable for building a BAC-contig. BAC libraries of both parental lines were constructed and BACs covering the QTL were isolated and sequenced by *Illumina*, *454*- and *PacBio*-technology. The reads were de novo assembled and contigs up to 120 kb were obtained. The contigs derived from the tolerant line were compared with those derived from the sensitive line. Surprisingly most of the sequences seemed to be identical between the tolerant and sensitive line. Hence the QTL could be diminished to an intervall of only 500 kb. Additional experiments were necessary to narrow down unequivocally the chilling tolerance QTL to a certain gene within the 500 kb. Hence, to complement this map-based cloning approach, the expression of the genes identified within this 500 kb region was analysed by REAL-time PCR. While most of these genes showed no expression differences between tolerant and sensitive lines, three of these genes showed chilling dependent expression differences in those lines. These results were confirmed by *Illumina* sequencing of RNA.

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P241

Transcriptomic analysis of senescence in maize inbred lines with different rate of senescence

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Behind the physiological and metabolic changes occurring at maize senescence there are changes in the expression of thousands of genes. We carried out a genomewide analysis of the changes in gene expression during leaf senescence in seven inbred lines of maize which differed in the rate of senescence. The lines were planted in a randomized completely block design with 2 replications in a single environment. Chlorophyll content and CO2 exchange were measured from flowering to complete senescence each 15 days in the middle part of the ear leaf of three plants per plot. Samples of the middle part of the ear leaf of three plants per plot were taken and mixed each 15 days from flowering to complete senescence. RNA library construction and sequencing were performed with TruSeq Stranded mRNA Library Prep Kit and HiSeq 4000 PE100 platform (Illumina Inc). Following reads alignment, annotation, and a differential expression analysis we detected 1083 and 588 genes that were up and down regulated, respectively, during the senescence in all seven lines. Because the genes were consistently detected in different lines we are confident in their involvement in senescence. However, some genes were detected in some lines, but not in others. For example, 1747 genes were detected only in 3 of the lines, indicating that the genes expressed at senescence partially depend on the specific lines. The genes that were down regulated were mainly involved in photosynthesis, while the genes up regulated were related to catabolic processes. 196 of the differentially expressed genes codified for transcription factors; some of them are homologous to transcription factors found in Arabidopsis in different signaling pathways, for example, ATAF1, GLK1, PIF5, JUB1, or AtNAP.

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P242

Turbocharging germplasm banks: genomic prediction goes into micro-world

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Effective evaluation of millions of genetic stocks is challenging, but it represents a great opportunity towards achieving global food security. Genomic prediction is a promising strategy to explore the potential of germplasm banks. Accurate predictions have been obtained for height, yield, and other macro-phenotypes. However, no studies have been reported on whether genome prediction is applicable for the micro-phenotype at the cellular level. Here, we tested the power of genomic prediction for nine maize traits measured from shoot apical meristem (SAM), which generates all above-ground organs. With 435,713 SNPs, we predicted the SAM cellular phenotypes for 2,687 maize diverse inbreds with models trained from 369 maize inbreds. The mean prediction accuracy from empirical validation with 500 inbreds reached 0.54, suggesting that genomic prediction can be applied for cellular phenotypes. Significantly higher prediction accuracies were further achieved by leveraging U statistics (upper bound of reliability) developed from genomic information alone. Our study expanded territories of genomic prediction for turbocharging germplasm banks from macro-phenotype space into micro-phenotype space.

Funding acknowledgement: National Science Foundation (NSF)

P243

Unleashing genetic diversity by increasing meiotic recombination : an *in silico* benchmark

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Genetic diversity is the fodder of genetic progress during selection processes, whether natural or artificial. Recombination during meiosis generates genetic diversity via the formation of crossovers which thus shuffle allelic combinations. Crossovers are heterogeneously distributed along chromosomes (hot and cold regions). This is important because for example in maize, 30% of the genes are in very cold regions and they arise with significant polymorphism. Selection can be impeded if advantageous alleles arise in such cold regions as they are thus difficult to extract or separate from nearby disadvantageous alleles.
We have been studying the influence of recombination rate, recombination landscape and other parameters (selection intensity, population size, genetic architecture such as the distribution and effects of QTLs) on the behavior of genetic gains and diversity when populations are subject to recurrent selection in breeding programs. Our approach is based on simulations using a quantitative genetics framework. We focus on plant breeding in maize, barley, cacao and Arabidopsis, investigating different ways of modifying recombination according to what is known to be possible experimentally today: modification of the genome-wide recombination rate (HyperRec technology), enhancement of recombination in cold pericentromeric regions (e.g., in the context of triploids), and targetting hot spots to specific parts of the genome.
To extract information about the behavior of the population througout *in silico* breeding programs, we measure different observables, including genetic gain, genetic diversity, linkage disequilibrium and also the coupling/repulsion status between loci. With our simulations, we identify how the different breeding choices and scenarios for modifying recombination influence the genetic gains achieved across successive generations.

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P244

Utilizing GWAS Results to Preferentially Treat Genomic Markers in Prediction Model

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Some of the most important agronomic crop traits are complex and thus governed by many genetic components of various effect sizes. In order to translate this complex genomic variability into genetic gain and crop improvement as effectively as possible, there is a critical need to explore the performance of variations on the basic genomic selection (GS) approach across a wide variety of genetic architectures. Using simulated data, we evaluated the performance of an approach that augments the Ridge Regression Best Linear Unbiased Prediction (RR-BLUP) GS model with fixed effect markers from a genome-wide association study (GWAS) conducted on a training set. Previous work in maize and rice suggest that this model should be ideal for predicting traits that have several large-effect genetic components, as well as many more components of smaller effect. We expand upon this work by evaluating traits simulated under a diverse set of genetic architectures from the "Goodman-Buckler" maize diversity panel. For each simulated trait, we evaluated the prediction accuracy of RR-BLUP models that included various numbers of peak-associated GWAS SNPs as fixed effect covariates. The results indicate that for certain genetic architectures, setting a preferential subset of markers from GWAS as fixed effects improved prediction accuracy. Expansion of this work will have implications in maize breeding as researchers continue to explore the utilization of the various types and amounts of data becoming readily available.

P245

ZmAuxRP1, encoding an auxin-regulated protein, coordinates the balance between growth and defense in maize

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To optimize fitness, plants must efficiently allocate their resources between growth and defense. Although phytohormone crosstalk has emerged as a major player in balancing growth and defense, the molecular basis by which plants manage this balance remains largely elusive. Previously, we identified a quantitative disease-resistance locus, *qRfg2*, in maize against *Gibberella* stalk rot. Through map-based cloning, we here demonstrate that *ZmAuxRP1* is the causal gene at *qRfg2*. *ZmAuxRP1* encodes a plastid stroma-localized auxin-regulated protein, which negatively regulates maize resistance to *Gibberella* stalk rot and *Fusarium* ear rot, but positively root growth. *ZmAuxRP1* is primed for instant response to pathogen challenge by implementing a rapid yet transient reduction of its expression to facilitate defense-oriented reprogramming of the transcriptome. ZmAuxRP1 promotes the biosynthesis of indole-3-acetic acid (IAA) and synchronically suppresses the formation of benzoxazinoids, which are potent defensive compounds in maize innate immunity. ZmAuxRP1 presumably acts as an integral regulator to modulate indole-3-glycerol phosphate (IGP) and/or indole flux at the branch point between IAA and benzoxazinoid biosynthetic pathways. The concerted interplay between IAA and benzoxazinoids can timely and efficiently regulate the growth–defense balance to optimize plant fitness.

Gene / Gene Models described: *ZmAuxRP1*; GRMZM2G063298
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P246

ZmCCT9 enhances maize adaptation to higher latitudes

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From its tropical origin in southwestern Mexico, maize spread widely over a wide latitudinal cline in the Americas. This feat defies the rule that crops are inhibited from spreading easily across latitudes. How the widespread latitudinal adaptation of maize was accomplished is largely unknown. Through positional cloning and association mapping, we resolved a flowering time quantitative trait locus (QTL) to a Harbinger-like transposable element positioned 57 kb upstream of a CCT transcription factor (*ZmCCT9*). The Harbinger-like element acts in cis to repress *ZmCCT9* expression to promote flowering under long days. Knockout of *ZmCCT9* by CRISPR/Cas9 causes early flowering under long days. *ZmCCT9* is diurnally regulated and negatively regulates the expression of the florigen *ZCN8*, thereby resulting in late flowering under long days. Population genetics analyses revealed that the Harbinger-like transposon insertion at *ZmCCT9* and the CACTA-like transposon insertion at another CCT paralog *ZmCCT10* arose sequentially following domestication and were targeted by selection for maize adaptation to higher latitudes. Our findings help explain how the dynamic maize genome with abundant transposon activity enabled maize to adapt over 90 degrees of latitude during the pre-Columbian era.

Gene / Gene Models described: *ZmCCT9*; GRMZM2G004483
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P247

ZmCOP II controls oil content in maize kernel

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Maize oil is highly valued as a resource of animal feed as well as vegetable oil for human consumption. Increasing the kernel oil content not only improves its nutritional value, but also leads to the increase in metabolizable energy. We previously identified *ZmCOP II*, encoding coat protein complex II (COPII), significantly associated with oil content in maize kernel by genome-wide association analysis. Whereas, it remains unknown how *ZmCOP II* regulates oil content. In this study, we validated the function of *ZmCOP II* by generating overexpression transgenic maize plants and CRISPR-Cas9 mediated null mutants. As expected, overexpressing *ZmCOP II* decreased oil content while knocking out of *ZmCOP II* increased oil content. This result indicate *ZmCOP II* negatively regulate oil accumulation in maize kernel. To further elucidate the molecular mechanism of *ZmCOP II* regulating oil accumulation, multiple experiments, such as expression analysis, yeast-two hybrids, and etc., were going on.

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P248

A single gene knock-out resource for maize: filling gaps in the genome with targeted *Ds-GFP* insertions

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The availability of a mutant line in which a single gene has been disrupted gives biologists a powerful tool in understanding that gene's action. Thus, sequence-indexed collections of single insertions are critical resources for elucidating gene function in organisms with a sequenced genome. Our NSF-PGRP-funded project is generating and sequence-indexing a collection of *Ds* transposon insertions in transgenic maize by taking advantage of next-generation sequencing (NGS) technologies. Specifically, our goals are to: (1) Assemble a set of 120 roughly equidistant *Ds** launching platforms carrying a GFP marker that allows simple visual selection of element transposition from any region of the genome; (2) Sequence-index several thousand *Ds** insertion sites from dozens of model platforms by NGS of 3-dimensional DNA pools on an Illumina MiSeq platform and data deconvolution with our InsertionMapper pipeline tool; and (3) Place all relevant information in our web-searchable database of insertion site sequences (http://acdsinsertions.org) cross-referenced to stocks available from the Maize Genetics Stock Center. At present, 86 launching platforms have been mapped to all 20 chromosome arms of the maize genome. Along, we have mapped 14,184 transposed *Ds** target sites to the reference B73 genome with the help of InsertionMapper. All the lines are listed in our database http://acdsinsertions.org, and 10.155 have been already sent to the Maize Genetics Stock Center for distribution. Future plan: to sequence-index Ds transpositions from chromosome arms that are currently not well covered. Our objectives are: (1) To generate a collection of 700 *Ds** transpositions from each of 8 *Ds** launching platforms located in poorly covered chromosome arms. (2) To sequence-index the additional collection of 5,600 new *Ds** insertions by NGS of 3-D DNA pools. Together with the >14,000 transposant stocks currently at hand, this new collection will complete the *Ds**-based single-gene knockout resource for maize.

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P249

Accuracy of the UniformMu resource is improved 15% by mapping Mu insertions in its native W22 genome

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The UniformMu National Public Resource for transposon-induced mutants includes 72,000 germinal insertions mapped in 14,024 seed stocks that are distributed to maize researchers world-wide by the Maize Coop Genetics Stock Center. UniformMu was constructed in a W22 (ACR) inbred background also used for Ac/Ds transposon resources. Mu insertions in UniformMu were initially mapped using the reference B73 genome. The high-quality W22 genome assembly created by the W22 Sequencing Consortium (www.maizegdb.org/genome) enabled re-mapping of insertions in the UniformMu genome. Of 68,866 Mu insertions assigned unique locations in the W22 genome, locations of 10,041 insertions (14.6%) differed in W22 and B73. These included 4,660 insertions found only in W22 showing that use of the W22 reference genome increased the total number of mapped insertions by nearly 7%. A similar number of insertions (4,865, 7.1%) were detected in both genomes, but assigned to different chromosomes. By contrast, less than 1% of insertions that mapped to the same chromosome in both genomes were displaced by greater than 10 Mb. The prevalence of differences in chromosome assignment suggested that sub-fractionation of duplicate genes contributes to divergent map locations in W22 and B73. Consistent with this hypothesis, misassigned insertions were enriched in three well-characterized, syntenous regions associated with whole-genome duplication in maize evolution: Chr 9L - Chr 1S; Chr 10L - Chr 2S; Chr 5S - Chr 1L. In each case, misassigned insertions showed a clear bias for one chromosome segment of each pair. While this pattern indicated biased fractionation, the expected bias favoring conservation of sub-genome A sites in the B73 genome occurred in only two of three duplications. This pattern suggests that biased fractionation operates at a regional level rather than on a sub-genome-wide basis. By eliminating mapping errors due to inbred differences, the new W22 genome sequence improves reliability of the UniformMu resource.

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P250

Atypical transposable element copies predict functional consequence

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Despite being genomic parasites, transposable elements (TEs) dominate the genomic landscape of maize, contributing the vast majority of DNA sequence. Although TEs disrupt the genomic environment they land in, generating mutations and altering epigenetic signatures of adjacent sequences, they have overtaken the genomes of all *Zea*. The negative effect of TEs on host fitness is mitigated by the separation of islands of genes in seas of transposons throughout the maize genome, such that genic euchromatin is limited in its interaction with TEs. But clear cases exist where these boundaries break down. For example, the maize allele of teosinte branched 1 (tb1) selected during domestication harbors a Hopscotch LTR retrotransposon insertion 65 kb upstream of the gene's coding sequence, and was selected for during domestication due to its impact on phenotype. This TE copy is distinguished from related copies throughout the genome from the same family by high levels of CHH methylation, and presence in a high recombination region, despite being far from genes.
To identify such aberrant TEs, without any prior knowledge of selection, we used features of the TE itself and the genomic environment it inserted into to develop expectations of how long natural selection would maintain each TE allele in maize populations. We identified thousands of TEs that have avoided removal by selection despite containing features differentiating them from related copies. By imputing inheritance of regions of the genome encompassed by these TEs to NAM RIL progeny, we find these regions disproportionately contribute to phenotypic variation, and are found near genes with tissue specific expression. We prioritize these outlier TEs for their contribution to molecular and phenotypic differentiation between maize lines. These analyses and future work to distinguish whether these TEs are polymorphic between maize lines will facilitate prediction of their consequences.

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P251

Buried treasures: the maize transposable elements *Dotted* and *Mrh*

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Transposable elements (TEs) are the major components of most sequenced genomes. Over 90% of the maize genomic DNA sequences are TEs and TE-derivatives. Among them, various families of class II transposons (DNA transposons) play a major role in shaping the genome. Most of them are nonautonomous driven by autonomous transposons whose presence and interactions are usually revealed genetically.
Dotted (*Dt*) was the first maize element characterized genetically as causing mutations at another locus, prior to its recognition as a TE. We are attempting to isolate it because of its enormous historic importance in the development of the concept of controlling elements. From a segregating population of the *Dt/a1-rDt* two-element system, we have cloned sequences amplified with primers based on the sub-terminal regions of its receptor element *rDt*. Sequence analysis revealed the presence of candidate *Dt* elements encoding a conserved *hAT* family dimerization domain-containing protein. We are in the process of identifying which of these corresponds to Rhoades' classic *Dt* element.
The autonomous transposon *Mrh* is known genetically to regulate its receptor element *rMrh* at the *A1* locus. The 80-bp terminal inverted repeats of *rMrh* are 70% identical with *Jittery*'s over the first 50 bp, so, like *Jit*, *Mrh* most likely belongs to the *Mutator* superfamily. We have amplified *Mrh*-related sequences using primers based on the *rMrh* terminal inverted repeats. Sequence analysis indicates a high conservation (more than 85% identity) of the encoded *Mrh* transposase to the known JITA transposase of *Jittery*, the second cloned autonomous element of *Mutator* superfamily. We have examined the possible genetic interaction between *Jittery* and *Mrh* and have shown that *Jittery* is able to trans-activate the *rMrh* element at the *A1* locus, as *Mrh* does. Our on-going efforts are to investigate how transposon specificities originate within a superfamily, an important issue in transposon evolution.

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P252

Characterization and mapping of the *required to maintain repression10* locus affecting paramutation

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Paramutation at the *Pl1-Rhoades (Pl1-Rh)* allele of the *purple plant1 (pl1)* locus results in meiotically heritable changes in gene regulation that are influenced by trans-homolog interactions[1]. Plants with a fully-expressed *Pl1-Rh* allele exhibit dark anther coloration, while plants with a repressed paramutant derivative (denoted *Pl'*) lack anther coloration. The transcriptional and post-transcriptional repression of *Pl1-Rh* is facilitated by *required to maintain repression* (*rmr*) factors[2]. We previously reported on eight different *rmr* factors identified by mutations induced with ethylmethanesulfonate (EMS) pollen treatment. At least six of these factors are required for the biogenesis of 24-nucleotide sRNAs[3,4,5,6,7,8] that may direct *de novo* cytosine methylation.
Genetic complementation tests and molecular mapping were used to determine that two recessive mutations, ems062986 and ems073240, define a novel locus provisionally designated *rmr10*. Results of genetic tests with the ems062986 mutation indicate that normal rmr10 function is required for the meiotic maintenance of *Pl'* states but not for facilitating paramutations at the *booster1* locus. RNA-seq data and a bioinformatics pipeline[9] are currently being used to identify likely candidate genes, and the effects of *rmr10* function on *pl1* RNA levels is being determined using qRT-PCR. Bulk low molecular weight RNA profiles will also be evaluated to characterize possible effects on small RNA accumulations. Results of these efforts expand our understanding of basic mechanisms controlling meiotically-heritable changes in gene regulation.

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P253

Characterization of polymorphic transposable element content between maize inbred lines

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Extensive transposable element (TE) variation has been shown between maize inbred lines on a loci-by-loci basis. However, genome-wide documentation of TE variation between inbred lines and consequences on transcriptional, epigenetic, and phenotypic variation has not previously been possible. With *de novo* assemblies of multiple maize inbred lines and de novo annotation of TEs within those assemblies now available, we are now poised to address this question on a genome-wide scale. Utilizing independent annotations of TEs in the B73, PH207, and W22 de novo genome assemblies, a detailed analysis of polymorphic TEs was conducted. A computational approach that utilizes sequences flanking TE insertions as well as anchor points between the three genomes has been developed to identify both non-nested and nested TE insertions unique to each genome. Preliminary analyses demonstrated high rates of TE polymorphism and showed the rate of polymorphism varies with respect to TE class. Future efforts will focus on identifying the phenotypic consequences of variable TE insertions and their potential influence on transcriptional and epigenetic variation within maize.

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P254

Cold induces transcriptional and methylation changes in the sensitive line B73

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In Northern Europe, maize development leads to high financial and environmental drying costs at harvest. Early sowing has been proposed as a strategy to overcome this problem, but this cycle shift subjects plants to cold in the first phase of their development. Such an early and prolonged cold affects maize metabolism, leading to yield reduction. Origin of this physiological modification is not well understood, and detection of candidate genes using genetic-based studies is challenging. Whether DNA methylation changes are involved in this phenotype remains to be elucidated.

Here, we analyze the impact of cold on methylome and transcriptome of the sensitive line B73. After 21 days of cold treatment, this genotype shows a chlorotic phenotype, as well as a delay in development. A total of 6937 genes were differentially expressed genes between "Cold" and "Standard" plants, with a slight majority of downregulated genes. Gene ontology analyses highlight an increase in DNA replication, DNA repair, and RNA processing functions and a decrease in photosystem activity. This is in accordance with the phenotype observed, and pinpoints that cold is indeed sensed as a physiological stress.

Cold induces genome-wide methylation changes, with an overall increase of methylation in "Cold" plants. Local modifications are also observed, with a total of 699 Differentially Methylated Regions (DMRs) between the two plant sets. The majority of these DMRs vary in both CG and CHG levels, and are located upstream and downstream of Transposable Elements (TEs). Another 30% varies only in CHG and is located in genes carrying TEs. A smallest fraction varies only in CG and is located in genes and away from TEs. These results suggest that cold induces both changes in TE spreading, regulation of TEs in genes, and gene body methylation.

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P255

Discovering the epigenetic memory of stress response in maize

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Stress perception and adaptation in plants require a variety of physiological, biochemical, transcriptional, and epigenetic responses. Dynamic changes in chromatin structure and concomitant transcriptional variations play an important role not only in stress response, but are also involved in epigenetic memory mechanisms. Histone marks and gene expression patterns could be indeed stably maintained during cell division and sexual reproduction, once the triggering stimulus has been removed. There is good evidence that chromatin may play a pivotal role in somatic memory phenomena and although many progresses have been made in understanding chromatin modifications implicated in plant response to environmental triggering conditions, we are still far from connecting molecular genetics and developmental data around environment and chromatin.
In order to understand whether environmental memories are created and eventually propagated, we integrated transcriptional and epigenetic data from maize plants subjected to a mild and prolonged drought stress and after the complete recovery from the stress. We observed that extensive transcriptional changes present soon after the stress application were only partially reset after the recovery stage. Concomitantly, ChIP-Seq analyses revealed a direct correlation between transcriptional variation and H3K4me3 or H3K9ac histone modification enrichment at the majority of stress-regulated gene loci. The facultative heterochromatin mark H3K27me3 was instead associated to a few developmentally regulated genes misregulated by the applied stress. In addition, several stress-responsive genes in which histone marks variations persist after the recovery stage were identified, indicating a form of stress memory.
Based on the emerged fundamental role of epigenetic mechanisms in regulating stress response and adaptation we are further evaluating the identified targets in the progeny of the stressed plants and in plants subject to repeated stress pressure

P256

Distinct pattern of DNA methylation in different subnucleosomal domains

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DNA methylation and nucleosome positioning are two essential epigenetic modification of most eukaryotic genome. However, the specific relationship between DNA methylation and nucleosome positioning has remained elusive, particularly, methylation pattern inside of octamer nucleosomes is not been investigated. Here we report a comprehensive survey of methylation variation in subnucleosomal level, and found the variation was mainly reflected by the difference between the central and peripheral domains of nucleosomes, which are corresponded to DNA bound to (H3-H4)2 tetramer and H2A-H2B dimers, respectively. Overall, CG methylation is enriched at central domain for nucleosomes in transposable elements and exons, but is enriched at peripheral domain for nucleosomes in intergenic regions. By contrast, CHG and CHH methylation tends to occurred at peripheral domain in most cases. Subnucleosomal DNA methylation pattern is cooperatively modulated by the activities of different methyltransferases, which DRM1/2 and CMT2/3 preferentially mediate the methylation at central and peripheral domain, respectively. Subnucleosomal DNA methylation pattern is also affected by demethylation process, including DME-mediated demethylation, which preferentially happened at central domain. In general, our results demonstrated the variation and regulation of DNA methylation in different subnucleosomal domains for the first time, which is helpful for further understanding of the function of DNA methylation.

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P257

Documenting the role of transposable elements in DNA methylation variation in maize

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A majority of the maize genome is comprised of transposable elements (TEs). These TEs can influence chromatin state and expression of nearby genes. Previous studies have documented numerous differences in DNA methylation among maize inbred lines. However, it has been difficult to determine whether these differences in DNA methylation are purely epigenetic or the result of nearby changes in DNA sequence. The availability of de novo genome assemblies for B73, PH207 and W22 provides an opportunity for a genome-wide analysis of the role of TEs in DNA methylation variation. Deep whole genome bisulfite sequencing (WGBS) data was generated for these three genotypes and used to identify thousands of differentially methylated regions (DMRs). These include DMRs that have alterations in CG, CHG or CHH methylation as well as various combinations of these context-specific changes. Many of these DMRs are located within or near TEs. Comparisons of the genome structure and TE annotation of B73, PH207 and W22 allowed the identification of a set of conserved and polymorphic TE insertions. The DNA methylation patterns flanking conserved TE insertions were assessed to determine whether the spreading of DNA methylation from TEs is consistent among genotypes. The regions flanking polymorphic TEs were analyzed to document the prevalence of DMRs resulting from a TE insertion. These analyses reveal many examples in which TEs influence DNA methylation of nearby sequences. Future work will focus on understanding how these changes in DNA methylation triggered by TEs could influence expression of nearby genes.

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P258

Identification and characterization of regulatory sequences in *Zea mays*

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Correct temporal and spatial regulation of gene expression is crucial for the successful development of an organism. Regulation of gene expression is in part accomplished through the coordinated action of cis-regulatory elements such as transcriptional enhancers. Whereas regulatory sequences are still poorly characterized in plants, they have been extensively characterized in mammals. Active enhancers are for example found to be associated with specific features such as particular histones marks, chromatin accessibility, low DNA methylation, presence of enhancer-specific transcripts and the ability to physically contact their target via the formation of chromatin loops.
Our study aims at a better identification and characterization of active enhancers in *Zea mays*. By making use of published bisufite-seq data sets and newly generated DNAseI-seq, ChIP-seq and RNA-seq data sets, about 1,500 putative regulatory sequences have been identified in young seedling and husk tissue. These include known and experimentally validated enhancers in maize. The enhancer candidates are characterized by low DNA-methylation, increased chromatin accessibility, and, unlike in animal systems, an asymmetric enrichment of H3K9ac. Tissue-specificity of enhancer candidates was defined based on the tissue(s) they were identified in. Currently, enhancer candidates are characterized in more detail.

Weber et al. (2016) Trends in Plant Science 21, 974. doi: 10.1016/j.tplants.2016.07.013.
Oka et al. (2017) Genome Biology 18:137. doi: 10.1186/s13059-017-1273-4.

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P259

Identification of new maize (root) mutants by Mu-seq

(submitted by Caroline Marcon <marcon@uni-bonn.de>)
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The well-described Mutant-seq (Mu-seq) approach enables the development of sequence-indexed reverse genetic mutant databases in maize (McCarty et al., 2013, Liu et al., 2016). Mu-seq simultaneously enables genome-wide identification and mapping of new Robertson´s *Mutator* insertion sites in individual F_2-families of large mutagenized maize populations. We generated Mu-seq libraries by crossing an active *Mutator* line into the inbred lines B73 and Co125. The inbred line Co125 was selected because it is representing a genetic resource for central European maize research due to its stable growth properties under temperate climatic conditions. After self pollinating the F_1-generation, segregating F_2-families were introduced in the Mu-seq pipeline. In our initial Mu-seq library, consisting of 576 F_2-families in B73 genetic background, 20,081 germinal insertions representing 11,457 different gene models were detected. As a proof of concept, a new mutant allele of the *roothair defective 3* (*rth3*) gene was identified in one of the mutagenized families. The mutant *rth3* is affected in root hair elongation (Wen and Schnable, 1994; Hochholdinger et al., 2008). Furthermore, forward genetic screens of the mutagenized families, in germination paper rolls, enable the identification of seedling mutant phenotypes 10-12 days after germination. Among other phenotypes, screening of ~25% of the 576 lines identified a striking dwarf mutant affected in root and shoot development. To identify genes identified in this forward genetic screen bulked segregant RNA-seq (BSR-seq) analyses (Liu et al., 2012) will be performed.

P260

Maize centromeres expand in the large genome background of Oaxaca and Zea. luxurians

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Little is known about how centromere size and location are determined. Previous work in maize has revealed that centromere size among grass species is highly correlated with total genome size, and maize centromeres expand when transferred into a larger genome background of oat. However, whether centromeres will expand if we intentionally enlarge the genome size of a specific species is still unknown. Maize is a model organism for investigating these topics because of its genetic resources, including extreme diversity of centromere types between homologous chromosomes. In this study, we test the hypothesis that centromere size positively correlates with genome size in maize species by introducing the chromosomes of B73 into Oaxaca and Zea.luxurians background plant, both Oaxaca and Zea.luxurians have a larger genome than B73. To increase the genome size while keeping centromere 2 and 5 homozygotes from B73, we have crossed B73 with Oaxaca and Zea.luxurians for several times. The genome size of these different lines have been estimated by the amount of DNA in cell nuclei from flow cytometry. The results showed that the genome size is bigger in the hybrids of B73 X Oaxaca and B73 X Zea.luxurians than in B73. The CENH3-ChIP experiment results suggested that four centromeres (centromere 2, 3, 8, 9) are expanded in the BC1F2 hybrids of B73 and Oaxaca, F2 of B73 and Zea.luxurians. These data provide new insights into the relationship between centromere size and genome size in maize species.

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P261

Parent-of-origin dependent nucleosome organization and its role on the regulation of genetic imprinting in maize endosperm

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Genomic imprinting confers allele-specific expression of genes depending on their parental origin. Nucleosomes, the fundamental units of chromatin, play a critical role in gene transcriptional regulation. However, it remains unknown whether differential nucleosome organization is related to the allele-specific expression of imprinted genes. Here we generated a genome-wide map of allele-specific nucleosome occupancy in maize endosperm and presented an integrated analysis of its relationship with parent-of-origin dependent gene expression and DNA methylation. We found that about 2.3% of nucleosomes showed significant parental bias in maize endosperm. The parent-of-origin dependent nucleosomes preferentially exist as single isolated nucleosomes. Parent-of-origin dependent nucleosomes were significantly associated with the allele-specific expression of imprinted genes, with nucleosomes positioned specifically in the promoter of non-expressed alleles of imprinted genes. Furthermore, we found that most of the paternal specifically positioned nucleosomes (pat-nucleosomes) were associated with parent-of-origin dependent differential methylated regions, suggesting functional link between the maternal demethylation and the occurrence of pat-nucleosome. Maternal specifically positioned nucleosomes (mat-nucleosomes) were independent of allele-specific DNA methylation, but seem to be associated with allele-specific histone modification. Our study provides not only the first genome-wide map of allele-specific nucleosome occupancy in plants but also a mechanistic connection between chromatin organization and genetic imprinting.

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P262

Reduction of DNA methylation during early embryogenesis enhances growth heterosis of maize plants

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Heterosis is the superior performance of heterozygous hybrid offspring compared to the parental homozygous inbred lines. It has been widely hypothesized to be associated with epigenetic modifications such as DNA methylation. The aim of this work was to test whether DNA methylation patterns established during early embryogenesis impacts upon the heterotic response. We treated maize embryos of inbred lines and hybrids with 5-aza-2'-deoxycytidine (aza), a DNA methyltransferase inhibitor. The treatment was restricted to one application of aza starting one day after pollination by using an in vitro embryo sac culture system. DNA methylation analysis by Reduced Representation Bisulfite Sequencing (RRBS) showed natural and artificially induced methylation dynamics between developmental stages of maize. The successful demethylation of symmetric CG and CHG contexts by the early aza-treatment was confirmed in 7 day-old embryos and in seedlings of inbred lines. Thus, hypomethylation of DNA was induced in the early embryo and maintained to the seedling stage. Growth rates of inbred and hybrid seedlings were used to determine the effect of methylation inhibition on this key heterotic trait. Comparing plants derived from aza-treated embryos and their untreated control group demonstrated a significant increase of the growth heterosis upon demethylation. In contrast, a later aza application to 14 day-old embryos affected growth in general, but not growth heterosis. Together our results indicate that DNA methylation patterns that establish during early maize embryogenesis have a negative effect on growth heterosis formation at developmental stages after germination.

P263

RNA polymerase IV contributes to hybrid vigor

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Maize RNA polymerase IV (RNAP IV) affects genome-wide RNAP II-based transcription[1] and is responsible for defining tissue-specific expression patterns of specific alleles[2,3]. RNAP IV also mediates and maintains transcriptional repression of alleles undergoing paramutation[4] – a meiotically-heritable change in gene regulation facilitated by *trans*-homolog interactions[5]. Paramutation behaviors of certain *red1* and *purple plant1* alleles controlling anthocyanin pigment production provide clear examples of single locus heterosis[6,7], leading to speculations that similar behaviors contribute to hybrid vigor[7,8]. Here it is reported that absence of RNAP IV function in B73 inbred parents impacts the heterotic traits of B73 / Mo17 hybrids. Hybrid gains in both plant height and grain yields are contributed by RNAP IV function in parental sporophytes. Understanding the genomic features that recruit RNAP IV and the various roles both RNAP IV and RNAP IV-generated 24 nucleotide RNAs play in defining RNAP II transcription patterns promises novel opportunities for predicting and controlling biomass production. Indeed, the differences between uniting gametes responsible for heterosis need not be Mendelian in nature[7,9].

1. Erhard *et al.* 2015 *Genetics* | 2. Parkinson *et al.* 2007 *Dev Biol* | 3. Erhard *et al.* 2009 *Science* | 4. Erhard *et al.* 2013 *Plant Cell* | 5. Hollick 2017 *Nat Rev Genet* | 6. Styles and Brink 1969 *Genetics* | 7. Hollick and Chandler 1998 *Genetics* | 8. Kermicle and Alleman 1990 *Development* | 9. Shull 1948 *Genetics*

Gene / Gene Models described: *rpd1*; GRMZM2G007681
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P264

Single-pollen sequencing for the study of novel meiotic phenotypes

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Meiotic recombination is a fundamental evolutionary driver and an indispensible tool for agricultural breeding. Advances in next-generation sequencing, coupled with genotyping by sequencing approaches, have allowed for the development of large-scale, population level assessments of crossover frequency and localization, as well as the construction of high-density linkage maps for quantitative trait mapping. Despite these advances, unbiased, genome-wide study of recombination in meiotic mutants remains difficult. The computational complexity of identifying crossovers in hybrid populations, constraints on the availability of plant material, and variable contributions of both the maternal and paternal genomes all pose significant challenges. Here, we describe the adaptation of single-cell DNA sequencing methodologies to individual maize pollen grains. Sequencing of pollen grains from biparental hybrid populations, coupled with statistical modeling of recombination breakpoints, allows for genome-wide, high-resolution mapping of crossover intervals. Using this approach, the distortion of patterns of recombination in DNA methyltransferase mutants is being characterized.

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P265

The maize *Ufo1* mutant results from ectopic over expression of an endosperm specific gene

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The maize mutant *Unstable factor for orange1* (*Ufo1*) is a dominant modifier of tissue specific expression of *pericarp color1* (*p1*). Thus *p1* has been used here as a reporter for the presence/absence of *Ufo1*. The common feature of *Ufo1-p1* interactions is that several epigenetically silent *p1* alleles are up regulated. Our global gene expression studies show that *Ufo1* up-regulates genes enriched for GO categories for response to various stimuli such as ROS and high light intensity. Genes involved in DNA replication and ribosome biogenesis are down regulated. The overall picture is that *Ufo1-1*. plants show elevated levels of stress under normal growth conditions. Using transcriptomic data we identified a candidate gene (named *G7*) in the mapping region. In wild type, *G7* is specific to endosperm but in *Ufo1-1* mutant it is ectopically overexpressed 45 to 200 fold higher in the three tissues used for RNAseq. Using PacBio long read assembly, we found that in Ufo1-1, *G7* has a *CACTA* insertion within the first intron. Regulation of expression of *G7* by the DNA methylation status of the CACTA transposon explains the incomplete penetrance and poor expressivity of *Ufo1-1*. Overexpression of *G7* explains pleiotropic defects as well as the dominance of *Ufo1-1* as confirmed by analysis of its silent epialleles.

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P266

The transposon landscape of the inbred W22

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The maize W22 genome has served as a foundation for maize genetics for nearly 60 years. Using short read sequencing technology we have assembled the genome and annotated the transposon content. We have also mapped an extensive collection of active *Dissociation* and *Mutator* transposons to the genome. Our analysis indicates over 177,000 that can be classified into nearly 27,000 families. Comparisons to the B73 genome revealed extensive heterogeneity in LTR and TIR composition and distribution between the two genomes. Of the approximately 250 *Mutator* insertions present in both genomes, only about half share a common insertion site. The others occupy unique positions, usually within 500 bp of a gene, raising the possibility of extensive inbred-specific cis-regulatory variation on gene expression. By mapping *Dissociation* and *Mutator* insertions mobilized in the W22 genome to this reference genome, we were able to accurately position approximately 10,000 previously unmapped *Mu* insertions and over two hundred *Ds* insertions. In summary, this high quality W22 assembly provides a foundation for comparative genomics in maize and an important resource for functional genomics.

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P267

Using chromatin features to identify and understand intergenic transcriptional regulatory elements in maize

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Transcriptional cis-regulatory elements (CREs) modulate the expression of genes via the action of sequence-specific DNA binding factors. CREs confer specificity for when and where genes are expressed during development and in response to stimuli. The importance of characterizing and cataloging the regulatory elements of a genome is akin to the importance of cataloging the protein-coding regions. However, identifying CREs poses a challenge because (1) they may act on target genes in an orientation- and distance-independent manner, and (2) sequence conservation may be limited to small transcription factor binding sites. The limitation of sequence-based methods necessitates the incorporation of epigenomic features for identifying CREs. Specifically, the structural and biochemical features of chromatin may be used as a proxy for the presence of CREs. The chromatin signatures of human CREs have been characterized extensively and a minimum set of structural and biochemical features are often used to locate CREs. However, a set of predictive chromatin features has yet to be developed for CREs in plants. We have generated datasets on DNA accessibility (ATAC-seq), cytosine methylation (Methylc-seq), transcriptional status (RNA-seq and RNA PolII ChIP-seq) and histone N-terminal covalent modifications (ChIP-seq) in the model crop Zea mays. Preliminary analyses reveal an abundance of putative CREs in the gene-distal intergenic space. A small set of chromatin attributes can parse the gene-distal putative CREs into distinct groups that are enriched for either acetylated histone H3 or trimethylated H3K27. These two groups of putative CREs appear to regulate genes with distinct functions—i.e., acetylated CREs correlate with dynamically regulated, stimuli-responsive genes and H3K27me3 CREs correlate with developmentally regulated genes.

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P268

Using GRO-Seq as a tool to understand transcriptional regulation in maize

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Global Run-On Sequencing (GRO-Seq) reads identify nascent transcription occurring across the entire genome[1]. In maize, GRO-Seq has been used to define regions where transcription rates are dependent on RNA polymerase IV (Pol IV) function[2]. In Arabidopsis, Pol IV RNAs are processed into 24 nucleotide (24nt) sizes that facilitate RNA-directed DNA methylation (RdDM)[3]. It remains unclear in either maize or Arabidopsis whether Pol IV itself or Pol IV-dependent cytosine methylation affects Pol II function[4]. Comparisons of GRO-Seq profiles from *dicer-like3* mutants having virtually no 24nt RNAs and *rpd1* mutants having neither Pol IVa nor Pol IVb function should distinguish these two potential regulatory mechanisms. Current efforts to characterize and optimize the nuclei isolations and *in vitro* transcription reactions needed to create GRO-Seq libraries will be presented. Using hybridization of radiolabeled nascent RNAs with single-gene riboprobes, the effects of Sarkosyl (reported transcription initiation inhibitor[5]) are being evaluated and optimal elongation times are being determined. Nuclei isolated from various tissue types and developmental stages are also being evaluated for *in vitro* transcriptional competency and thus suitability for GRO-Seq library production. GRO-Seq profiles generated from nuclei treated with alpha-amanitin (Pol II-specific inhibitor) will potentially identify nascent Pol IV and/or Pol V transcripts. GRO-Seq profiles of specific mutants will also be compared to distinguish Pol II transcription specifically affected by Pol IVa or Pol IVb[6, 7]. Ultra-deep coverage of GRO-Seq reads will assist future genome annotations, including gene model validations, enhancer calls, defining 3' pretermination transcription and other intergenic regions of non-coding transcription.

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History of the Maize Genetics Conference

Year	Annual	Location	Dates	Chair
2018	60	Saint-Malo, France	March 22-25	Alain Charcosset
2017	59	St. Louis, Missouri	March 9-12	Erich Grotewold
2016	58	Jacksonville, Florida	March 17-20	David Braun
2015	57	St. Charles, Illinois	March 12-15	Mark Settles
2014	56	Beijing, China	March 13-16	Ann Stapleton
2013	55	St. Charles, IL	March 14-17	Phil Becraft
2012	54	Portland, OR	March 15-18	John Fowler
2011	53	St. Charles, IL	March 17-20	Erik Vollbrecht
2010	52	Riva del Garda, Italy	March 18-21	Jane Dorweiler
2009	51	St. Charles, IL	March 12-15	Steve Moose
2008	50	Washington, DC	February 27 - March 2	Thomas Brutnell
2007	49	St. Charles, IL	March 22-25	Anne Sylvester
2006	48	Asilomar, Pacific Grove, CA	March 9-12	Jay Hollick
2005	47	Lake Geneva, WI	March 10-13	Martha James
2004	46	Mexico City, Mexico	March 11-14	Mike Scanlon
2003	45	Lake Geneva, WI	March 13-16	David Jackson
2002	44	Kissimmee, FL	March 14-17	Sarah Hake and Sue Wessler
2001	43	Lake Geneva, WI	March 15-18	Torbert Rocheford and Sue Wessler
2000	42	Coeur d'Alene, ID	March 16-19	Rebecca Boston and Sue Wessler
1999	41	Lake Geneva, WI	March 16-19	Julie Vogel and Cliff Weil
1998	40	Lake Geneva, WI	March 19-22	Mike McMullen
1997	39	Clearwater Beach, FL	March 13-16	Paul Sisco
1996	38	St. Charles, IL	March 14-17	Paul Chomet
1995	37	Asilomar, Pacific Grove, CA	March 16-19	Karen Cone
1994	36	St. Charles, IL	March 24-27	Kathy Newton
1993	35	St. Charles, IL	March 18-21	Tim Nelson
1992	34	Asilomar, Pacific Grove, CA	March 19-22	Sarah Hake
1991	33	Lake Delavan, WI	March 21-24	Jim Birchler
1990	32	Lake Delavan, WI	March 8-11	
1989	31	Lake Delavan, WI	March 2-5	
1988	30	Madison, WI	March 25-27	
1987	29	Lake Delavan, WI	March 20-22	
1986	28	Lake Delavan, WI	March 21-23	Curt Hannah
1985	27	Lake Delavan, WI	March 29-31	Hugo Dooner
1984	26	Champaign, IL	March 10-11	Earl Patterson
1983	25	Allerton Park, IL	March 12-13	Earl Patterson
1982	24	Allerton Park, IL	March 13-14	Earl Patterson
1981	23	Allerton Park, IL	March 14-15	Earl Patterson
1980	22	Allerton Park, IL	March 8-9	Earl Patterson
1979	21	Allerton Park, IL	March 10-11	Earl Patterson
1978	20	Allerton Park, IL	March 11-12	Earl Patterson
1977	19	Allerton Park, IL	March 12-13	Earl Patterson
1976	18	Allerton Park, IL	March 13-14	Earl Patterson
1975	17	Allerton Park, IL	March 8-9	Earl Patterson

Year	Annual	Location	Dates	Chair
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

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