59th Annual Maize Genetics Conference

Program and Abstracts

March 9 – March 12, 2017

The Union Station
St. Louis, Missouri

This conference received financial support from:

National Science Foundation
USDA NIFA
DuPont Pioneer
Monsanto
Syngenta
KWS SAAT AG
National Corn Growers Association
BioNano Genomics
BASF Plant Science
Bayer Crop Science
Evogene
Dow AgroSciences
Benson Hill

We thank these contributors for their generosity!

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Cover image description

Second node of maize inbred Ky21 at maturity grown in a 50/50 mix of peat based soilless potting media and calcined clay at the Purdue Horticultural Greenhouse Facility, West Lafayette, Indiana.

Cover art by

Norman Best
Purdue University

General Information

Meeting Registration

Thursday: 3:00 PM to 9:30 PM: Depot Registration Office
Friday: 7:00AM to 1:30 PM: Depot Registration Office

Meals

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

Talks and Posters

All Talks will be presented in the Regency Ballroom.

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

The maize meeting is a forum for presentation and discussion of unpublished material.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

Hospitality

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

After 1 AM, the Missouri Pacific Room will be available for continued socializing. 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 2017 Steering Committee

Erich Grotewold, Chair.....(grotewold.1@osu.edu)
Alain Charcosset, co-Chair (alain.charcosset@moulon.inra.fr)
Gernot Presting ..... (gernot@hawaii.edu)
Petra Wolters..... (petra.wolters@cgr.dupont.com)
David Braun..... (braundm@missouri.edu)
Karen McGinnis ..... (mcginnis@bio.fsu.edu)
Jianbing Yan ..... (yjianbing@gmail.com)
Natalia de Leon..... (ndeleongatti@wisc.edu)
Sylvia Sousa..... (sylvia.sousa@embrapa.br)
Maike Stam ..... (m.e.stam@uva.nl)
Andrea Eveland ..... (aeveland@danforthcenter.org)
Thomas Slewinski ..... (thomas.l.slewinski@monsanto.com)

Ex officio:
Carson Andorf
Paula McSteen, Treasurer
Marty Sachs, Local Host

Acknowledgements

Many thanks go to John Portwood and Carson Andorf for their tremendous efforts in organizing, assembling, and advertising the conference program. We also greatly thank Angela Freemyer and her team at the University of Missouri Conference Office for helping to organize the conference, handling registration and dealing with a multitude of other issues. Special thanks are also extended to Barbara Koenig Brugger and the Union Station staff for their help in organizing this conference, and to Darwin Campbell and John Portwood for providing AV and other support. Thanks go to Thomas Slewinski and Erich Grotewold for their efforts in securing funding to support graduate student attendance at this meeting. Finally, many, many thanks go to Marty Sachs for his work as local organizer and for his wisdom in all things related to the Maize Meeting.

Useful Links

2017 Maize Meeting Website

http://maizegdb.org/maize_meeting/2017

2018 Maize Meeting Website (Available November 2017)

http://maizegdb.org/maize_meeting/2018

Abstract Book (Electronic version)

http://maizegdb.org/maize_meeting/abstracts/2017Program.pdf

Cover Image

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

The MaGNET Program and 2017 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.

2017 MaGNET Awardees

Undergraduate

Sarfo Kantanka, Florida A&M University	
Daniel Hayden, University of Oklahoma	
Brianna Griffin, Florida State University	Poster #144
Jason Peters, Southern University	

Graduate Student

Jonas Rodriguez, University of Wisconsin-Madison	
Janette Mendoza, University of New Mexico	Poster #221
Roberto Alers Velazquez, University of Toledo	
Aimee Uyehara, University of Hawaii at Manoa	Poster #128

Mentor Accompanying Student

Gokhan Hacisalihoglu, Florida A&M University	Poster #94
Katy Guthrie, University of Missouri-Columbia	Poster #72
Laura Morales, Cornell University	Poster #298
Subbaiah Chalivendra, Louisiana State University	Poster #293
John Gray, University of Toledo	Poster #67
Maria Angelica Sanclemente, University of Florida	Poster #107
Brett Burdo, University of Wisconsin-Madison	Poster #292
Angel Del Valle Echevarria, University of Hawaii at Manoa	Poster #189

The MaGNET program of the Maize Genetics Conference is supported by grant IOS-1659999 from the National Science Foundation.

Schedule of Events

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

Thursday, March 9

2:00 PM – 6:00 PM	**OPTIONAL PRE-CONFERENCE WORKSHOPS**	
2:00 PM – 3:30 PM	**"The Maize Genomes"**	Midway Suite 4
2:00 PM – 3:30 PM	**"Losing the Fear of the Command Line Part I**	Midway Suite 3
3:30 PM – 4:30 PM	**"The Carpenter Workshop: Maize, Missouri, and Meeting the Need"**	Midway Suite 4
3:30 PM – 4:30 PM	**"Career Opportunities"**	Midway Suite 3
4:30 PM – 6:00 PM	**"Maize Tools & Resources"**	Midway Suite 4
4:30 PM – 6:00 PM	**"Losing the Fear of the Command Line Part II"**	Midway Suite 3
	Pre-registration recommended for the above sessions.	
3:00 PM – 9:30 PM	**REGISTRATION** (Depot Registration Office)	
3:00 PM – 6:00 PM	**POSTER HANGING** (Midway)	
6:00 PM – 7:00 PM	**DINNER** (Midway)	
7:00 PM – 9:00 PM	**SESSION 1 – PLENARY TALKS** Chair: David Braun *(Regency Ballroom)*	
7:00 PM	**WELCOME AND ANNOUNCEMENTS**	(Regency Ballroom)
7:15 PM	**Nathan Springer, University of Minnesota** *Shedding light on the dark spaces of the maize genome*	
8:05 PM	**Rebecca Nelson, Cornell University** *Sacrificing for security? Trade-offs related to disease resistance in plants*	
9:00 PM – 1:00 AM	**INFORMAL POSTER VIEWING & HOSPITALITY** (Midway)	

Friday, March 10

7:00 AM – 8:00 AM	**BREAKFAST** (Midway)
7:00 AM – 1:30 PM	**REGISTRATION** (Depot Registration Office)
8:00 AM – 9:55 AM	**SESSION 2 – THE MAIZE GENOMES** Chair: Gernot Presting *(Regency Ballroom)* Talks 1-5.
8:00 AM	**ANNOUNCEMENTS** (Regency Ballroom)
8:15 AM	**Kelly Dawe, University of Georgia** [T1] *A novel maize kinesin causes neocentromere activity and meiotic drive, altering inheritance patterns across the genome*
8:35 AM	**Maud Tenaillon, Le Moulon – INRA, France** [T2] *Evolutionary genomics of European maize and its American counterparts*
8:55 AM	**Miguel Vallebueno-Estrada, Cinvestav, Mexico** [T3] *Genetic diversity and genomic constitution of ancient Tehuacan maize dated 5300 to 5000 years before present*
9:15 AM	**Tim Kelliher, Syngenta** [T4] *MATRILINEAL, a sperm-specific phospholipase, triggers maize haploid induction*
9:35 AM	**Alyssa Anderson, University of California, Berkeley** [T5] *Understanding maize development through the Liguless narrow mutant and its phenotypic modifier: Sympathy for the ligule*
9:55 AM	**BREAK**
10:45 AM – 12:25 PM	**SESSION 3 – THE GENES THAT MAKE MAIZE I** Chair: Karen McGinnis *(Regency Ballroom)* Talks 6-10.
10:45 AM	**Hannes Claeys, Cold Spring Harbor Laboratory** [T6] *Natural variation in meristem size regulation: a naturally occurring hypomorphic allele of* thick tassel dwarf1 *modifies the phenotype of* fasciated ear2 *and increases kernel row number in maize*
11:05 AM	**Silvio Salvi, University of Bologna, Italy** [T7] *Cloning of* Vgt3*, a major QTL for flowering time in maize*
11:25 AM	**Dean DellaPenna, Michigan State University** [T8] *Natural variation for Vitamin E content is controlled by a novel chlorophyll cycle in maize grain*
11:45 AM	**Stacie Shuler, University of Wisconsin-Madison** [T9] *Return to near-normal starch accumulation: the unexpected contribution of pullulanase in a* su1-ref *background*
12:05 PM	**Zhaobin Dong, University of California, Berkeley** [T10] tassels replace upper ears1 *encodes a BTB/POZ ankyrin repeat gene that is directly targeted by* teosinte branched1

Friday, March 10 (continued)

12:30 PM – 1:30 PM **LUNCH** (Midway)

1:30 PM – 5:00 PM **POSTER SESSION 1** (Midway)

1:30 PM – 3:00 PM *Presenters should be at odd numbered posters.*
3:00 PM – 4:30 PM *Presenters should be at even numbered posters.*

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

4:40 PM – 6:00 PM **SESSION 4 – THE GENES THAT MAKE MAIZE II**
Chair: Petra Wolters *(Regency Ballroom)* Talks 11-14.

4:40 PM **Mona Mazaheri, University of Wisconsin-Madison** [T11]
Zmm22 *gene in maize has pleiotropic effects on traits important for the production of food, feed, and fuel*

5:00 PM **Nelson Garcia, Rutgers University** [T12]
The maize dek34-Dsg *encodes Tti2, a member of the TTT complex essential for PIKK stability*

5:20 PM **Camila Ribeiro, University of Florida** [T13]
Engineering amyloplast 6-phosphogluconate dehydrogenase activity to improve heat stability of the oxidative pentose phosphate pathway

5:40 PM **Adrienne Moran Lauter, USDA-ARS** [T14]
Pectinmethylesterase (PME) is differentially expressed in Ga1-S *silks and maps to a PME-repeat region near the* Ga1-S *locus*

6:00 PM – 7:00 PM **DINNER** (Midway)

7:00 PM – 9:00 PM **SESSION 5 – MCCLINTOCK PRIZE PRESENTATION**
Chair: Shawn Kaeppler *(Regency Ballroom)*

7:00 PM **Shawn Kaeppler, MGEC Chair**
Early- and Mid-Career Awards

7:10 PM **Jim Birchler, University of Missouri**
McClintock Prize Presentation

7:30 PM **Michael R. Freeling, University of California at Berkeley** [M1]
Mutagenic consequences of polyploidy and how plant polyploids repeatedly avoided extinction and took over the world

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

Saturday, March 11

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

8:00 AM – 10:00 AM **SESSION 6 – EXPRESSING THE GENOME**
Chair: Andrea Eveland *(Regency Ballroom)* Talks 15-20.

8:00 AM **Qi Li, Chinese Academy of Sciences, China** [T15]
Maize imprinted gene floury3 *encodes a PLATZ protein required for tRNA and 5S rRNA transcription through interaction with RNA Polymerase III*

8:20 AM **Steffen Knauer, Cold Spring Harbor Laboratory** [T16]
An expression atlas of the maize shoot apex reveals signatures TFs as important drivers for cell identity and sub-functionalization of key downstream factors

8:40 AM **Prakitchai Chotewutmontri, University of Oregon** [T17]
Contribution of translational control to establishing the distinct proteomes of bundle sheath and mesophyll cells in maize

9:00 AM **Wenwei Xiong, Montclair State University** [T18]
Highly-interwoven communities of gene regulatory network unveil crucial genes for maize seed development

9:20 AM **Thomas Hartwig, Stanford University** [T19]
Functional analysis of genetic variation of the brassinosteroid cis-regulatory network using hybrid allele-specific ChIP-Seq

9:40 AM **Amy Siebert, Oakland University** [T20]
Evolutionarily conserved role of a novel RNA binding protein involved in the splicing of U12-type introns

10:00 AM – 10:45 AM **BREAK**

10:45 AM – 12:25 PM **SESSION 7 – COMMUNICATING WITHIN AND BETWEEN CELLS AND PLANTS**
Chair: Michael Muszynski *(Regency Ballroom)* Talks 21-25.

10:45 AM **Marisa Otegui, University of Wisconsin, Madison** [T21]
Regulation of vacuolar trafficking by autophagy in maize aleurone cells

11:05 AM **Rachel Wang, Academia Sinica, Taiwan** [T22]
The mitosis-to-meiosis transition of pollen mother cells is controlled independently by Mac1 *and* Am1 *in maize*

11:25 AM **Claudiu Niculaes, Technical University of Munich, Germany** [T23]
A combination of pharmacological approaches and genetics provides insights into benzoxazinoid exudation by young maize roots

11:45 AM **Alisa Huffaker, University of California, San Diego** [T24]
Comparative phosphoproteomic analysis of immunoregulatory signaling in maize and arabidopsis

12:05 PM **Xiquan Gao, Nanjing Agricultural University, China** [T25]
Crosstalk between 9-oxylipins and jasmonates pathways modulates the maize resistance to stem rot caused by Fusarium graminearum

Saturday, March 11 (continued)

12:30 PM – 1:30 PM **LUNCH** (Midway)

1:30 PM – 5:00 PM **POSTER SESSION 2** (Midway)

1:30 PM – 3:00 PM *Presenters should be at even numbered posters.*
3:00 PM – 4:30 PM *Presenters should be at odd numbered posters.*

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

5:00 PM – 6:00 PM **COMMUNITY SESSION - Maize Genetics Executive Committee**
MGEC Chair: Shawn Kaeppler (Regency Ballroom)

6:00 PM – 7:00 PM **DINNER** (Midway)

7:00 PM – 8:55 PM **SESSION 8 – PLENARY TALKS**
Chair: Alain Charcosset *(Regency Ballroom)*

7:00 PM **ANNOUNCEMENTS** (Regency Ballroom)

7:15 PM **Jane Glazebrook, University of Minnesota** [Plen 3]
Plant immunity - The signaling network and the role of a family of calmodulin-binding proteins

8:05 PM **Philip Becraft, Iowa State University** [Plen 4]
Regulation of endosperm development: Insights and surprises

9:00 PM – 2:00 AM **INFORMAL POSTER VIEWING/DANCE** (Midway)

Sunday, March 12

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

Posters should be taken down by 9 am!

8:20 AM – 9:50 AM **SESSION 9 – INTERACTIONS WITH THE ENVIRONMENT**
Chair: Ivan Baxter *(Regency Ballroom)* Talks 26-29.

8:20 AM **ANNOUNCEMENTS** (Regency Ballroom)

8:30 AM **Qin Yang, North Carolina State University** [T26]
A caffeoyl-CoA O-methyltransferase *gene confers quantitative resistance to multiple pathogens in maize*

8:50 AM **Davide Sosso, Stanford University** [T27]
Corn smut sugar pathophysiology detected by live FRET imaging

9:10 AM **Shaoqun Zhou, Cornell University** [T28]
Genetic control of maize biochemical defense against Fusarium graminearum seedling blight.

9:30 AM **Ross Zhan, Purdue University** [T29]
A forward genetics approach to explore natural variation for enhancer/suppressors identifies components of the guard strategy of plant immunity

9:50 AM **BREAK**

10:20 AM – 11:30 AM **SESSION 10 – EMERGING TOOLS & CHALLENGES**
Chair: Sylvia Morais de Sousa *(Regency Ballroom)* Talks 30-32.

10:20 AM **David Wills, USDA-ARS** [T30]
Testing hypotheses on adaptation with exotic allele series at photoperiod loci in maize

10:40 AM **Karl Kremling, Cornell University** [T31]
Revealing the regulatory impacts of rare variants in maize with low cost RNASeq

11:00 AM **Carolyn Rasmussen, University of California, Riverside** [T32]
Mathematical modeling predicts division plane orientation in maize cells

11:30 AM **ADJOURNMENT**

Posters

Computational and Large-Scale Biology

P1	**Ethalinda Cannon** <ekcannon@iastate.edu>	*The Gene Pages at MaizeGDB*
P2	**Hung Nguyen** <hnn5y7@mail.missouri.edu>	*MaizeMine: A new data mining warehouse for MaizeGDB*
P3	**Mary Schaeffer** <schaefferm@missouri.edu>	*Using MaizeGDB tools and interfaces to find Stocks*
P4	**John Portwood** <portwood@iastate.edu>	*MaizeGDB: New resources for maize researchers*
P5	**Lisa Harper** <lisaharper@me.com>	*AgBioData: A consortium of agricultural-related databases*
P6	**Keting Chen** <kchen@iastate.edu>	*Characterization of the surface lipid metabolic network for maize silks: Impact of genetics and environment*
P7	**Nan Jiang** <jiang.1359@osu.edu>	*A maize gene regulatory network for phenolic metabolism*
P8	**Kevin Schneider** <kevinls@hawaii.edu>	*Centromeric genes of maize*
P9	**Justin Sapp** <jts68@duke.edu>	*Characterizing the diversity of brace root architecture and anatomy in maize*
P10	**Dan Park** <Woojun.d.park.15@dartmouth.edu>	*Co-expression network implicates functional divergence of gene isoforms in maize*
P11	**Avimanyou Vatsa** <akvhxd@mail.missouri.edu>	*Computational issues in grouping complex phenotypes*
P12	**Philip Saponaro** <saponaro@udel.edu>	*Computer vision with macroscopic microscopy: automating the extraction of pathogenesis information for quantitative analysis*
P13	**Rajiv Parvathaneni** <rparvathaneni@danforthcenter.org>	*Defining gene regulatory networks controlling early inflorescence development in maize*
P14	**Li Wang** <lilepisorus@gmail.com>	*Detection of highland adaptation in maize landrace populations*
P15	**Alexandra Asaro** <aasaro@wustl.edu>	*Determination of gene expression in maize roots through RNA sequencing*
P16	**Chenyong Miao** <cmiao@huskers.unl.edu>	*Developing a new method for multiple species GWAS (MSG)*
P17	**Lang Yan** <langyan0807@hotmail.com>	*Developing transcriptomic resources for Tripsacum to study the adaptation of a maize relative to temperate climates*
P18	**Gu Wei** <guwei89711@foxmail.com>	*Dynamic transcriptome of maize ovule early development*
P19	**Sandra Unterseer** <sandra.unterseer@tum.de>	*European Flint reference sequences complement the maize pan-genome*
P20	**Colton McNinch** <cmcninch@iastate.edu>	*Evaluation of dynamic growth responses of maize hybrids via, high throughput, field-based time-lapse photography*
P21	**Anne Lorant** <alorant@ucdavis.edu>	*Evolutionary genetics of natural populations of teosinte*
P22	**Dae Kwan Ko** <dkko@msu.edu>	*Expansion of the wisconsin diversity panel to further document the maize pan-transcriptome*
P23	**Thomas Kono** <konox006@umn.edu>	*Fates of tandem duplications in the maize genome*

P24	**Zhikai Liang** <zliang@huskers.unl.edu>	*Field phenotype prediction on maize using novel phenomic tools and environmental information*
P25	**Cheng Huang** <hc66@cau.edu.cn>	*Functional characteristics of co-expression gene islands in maize genome*
P26	**Mingze He** <mhe@iastate.edu>	*G4 quadruplexes in and near regulatory elements of maize genes involved in tissue development and altered transcriptional response to abiotic stresses*
P27	**Daniel Vera** <vera@genomics.fsu.edu>	*Genomaize, a UCSC genome browser for maize genomes*
P28	**Bo Wang** <bwang@cshl.edu>	*Genome assembly of maize Ki11 genome using long-read sequencing*
P29	**Adam Johnson** <afj8c8@mail.missouri.edu>	*Global gene expression analysis of a dosage series of chromosome arm 1L*
P30	**Wilberforce Ouma** <wilberzach@gmail.com>	*GRASSIUS: Helping unravel the regulatory repertoire of the grasses*
P31	**Rumana Aktar** <rayy7@mail.missouri.edu>	*High throughput automatic mosaicing of the field from drone aerial imagery*
P32	**Abdalla Zanouny** <azibrahim@wisc.edu>	*High-throughput phenotyping detects large natural variation of maize growth at seedling and early juvenile stages, and its correlation with adult phenotypes*
P33	**Wenwei Xiong** <xiongwe@mail.montclair.edu>	*Highly-interwoven communities of gene regulatory network unveil crucial genes for maize seed development*
P34	**David Hufnagel** <davidehuf@gmail.com>	*Hybridization between parapatric teosinte populations results in the formation of three unique hybrid groups in Mexico*
P35	**Blaise Weber** <b.b.m.weber@uva.nl>	*Identification and characterization of distant enhancers in Zea mays*
P36	**Luis M. Avila** <lavila@ucdavis.edu>	*Identification of inversion polymorphisms from genomic data using a nonparametric Bayesian estimator*
P37	**Connor Long** <connor.long@doane.edu>	*Identifying indicators of cold tolerance in maize root exudate through NMR fingerprinting*
P38	**Daniel Laspisa** <dlaspisa@hawaii.edu>	*Improving maize centromere sequences of RefGen V4*
P39	**Robert Schaefer** <schae234@umn.edu>	*Integrating co-expression networks with GWAS to detect causal genes driving elemental accumulation in maize*
P40	**Shuai Zeng** <zengs@mail.missouri.edu>	*KBCommons: A multi 'OMICS' integrative framework for database and informatics tools*
P41	**John Hodge** <jgerardhodge@gmail.com>	*Landmark-based semi-automated phenotyping for developmental phenotypes*
P42	**Maria Mejia Guerra** <mm2842@cornell.edu>	*Machine learning approaches for data integration to model regulatory architectures*
P43	**Elizabeth Sampson** <samps225@umn.edu>	*Machine vision phenotyping of maize seedling growth and morphology*
P44	**Nathan Miller** <ndmiller@wisc.edu>	*Machine vision seedling emergence assay for maize seed biology*
P45	**Carrie Davis** <davisc@cshl.edu>	*MaizeCODE: From linear sequence to active regions of the maize genome*
P46	**Sidharth Sen** <ssz74@mail.missouri.edu>	*Mining auxin response factor binding sites in maize using informatics approaches*
P47	**Joshua Stein** <steinj@cshl.edu>	*Mining maize with Gramene*

P48	**Hao Wu** <haowu@iastate.edu>	*Nkd1, Nkd2 and Opaque2 play essential roles in gene regulatory network of maize endosperm development*
P49	**Fei-Man Hsu** <fmhsu0114@gmail.com>	*Optimized reduced representation bisulfite sequencing reveals tissue-specific mCHH islands of maize*
P50	**Daniel Wickland** <wicklan2@illinois.edu>	*Optimizing genotyping-by-sequencing for gene mapping in polyploids*
P51	**Wenbin Mei** <wbmei@ucdavis.edu>	*Population genomics of copy number variation in a natural population of teosinte*
P52	**Solmaz Hajmohammadi** <solmaz.hajmohammadi@lemnatec.de>	*Precision farming using high-throughput field phenotyping system*
P53	**Ian Braun** <irbraun@iastate.edu>	*Predicting cleavage activity of CRISPR-Cas9 across diverse maize germplasm using reference genome analysis*
P54	**Fabio Gomez-Cano** <gomezcano.1@osu.edu>	*Predicting novel gene regulatory interactions by combining different datasets*
P55	**Penghao Wu** <pw396@cornell.edu>	*QTL of 29 novel traits contributing to drought tolerance identified by both of linkage analysis and GWAS in tropical maize*
P56	**Brian Smith-White** <smtwhite@ncbi.nlm.nih.gov>	*RefSeq curation at NCBI - adding value to the maize genome resources*
P57	**Michael Campbell** <mcampbel@cshl.edu>	*Resources for maize genome annotation: Lessons learned from B73*
P58	**Tyr Wiesner-Hanks** <tw372@cornell.edu>	*RNA-seq of maize and the fungal pathogen Setosphaeria turcica*
P59	**Indrajit Kumar** <ikumar@danforthcenter.org>	*Role of translational dynamics during photosynthetic differentiation of maize leaf*
P60	**Xianjun Lai** <xlai3@unl.edu>	*Searching for parallel signatures of selection during domestication in maize and sorghum*
P61	**Nancy Manchanda** <nancym@iastate.edu>	*Sequencing, assembly, and annotation of B104, a maize transformation resource*
P62	**Yang Zhang** <yzhang91@unl.edu>	*Statistical approaches to identifying differentially regulated orthologs (DROs) across related grass species*
P63	**Yinping Jiao** <yjiao@cshl.edu>	*The complex sequence landscape of maize revealed by single molecule technologies*
P64	**Gen Xu** <jsycxugen@163.com>	*The history of maize domestication and adaptation as revealed by a genome-wide survey of SNP variation*
P65	**Adam Johnson** <afj8c8@mail.missouri.edu>	*The inverse effect across phyla*
P66	**Carolyn Lawrence-Dill** <triffid@iastate.edu>	*The maize Genomes to Fields (G2F) Initiative: Data management and availability*
P67	**John Gray** <jgray5@utnet.utoledo.edu>	*The Maize TFome - development of a transcription factor open reading frame collection for functional genomics*
P68	**Alex Brohammer** <broha006@umn.edu>	*The role of differential fractionation in maize genome content variation*
P69	**Kameron Wittmeyer** <ktw5072@psu.edu>	*The unique transcriptome and genome structure of maize Ufo1 mutant*
P70	**Natalie Nannas** <njnannas@uga.edu>	*Transformation and multimerization of large linear molecules via bombardment*
P71	**Sanzhen Liu** <liu3zhen@ksu.edu>	*Unbiased K-mer analysis reveals changes in copy number of highly repetitive sequences during maize domestication and improvement*

P72 **Katherine Guthrie** <klgdn2@mail.missouri.edu>
Uncovering new players in the auxin pathway through WGCNA analysis

P73 **Elena Rice** <elena.a.rice@monsanto.com>
Understanding variance of maize traits at multiple biological scales

P74 **Peter Bradbury** <pjb39@cornell.edu>
Using expression QTL with machine learning to learn about the usefulness of genomic annotations for finding causal variants

P75 **John Fowler** <fowlerj@science.oregonstate.edu>
Using genome-scale transposable element insertion datasets to investigate mutation in gametophytically-expressed genes

P76 **Marianne Emery** <marianneemery@mail.missouri.edu>
Using RNAseq to identify key player genes driving soybean drought response in high and low nitrogen fixing genotypes

P77 **Nathanael Ellis** <Nellis@danforthcenter.org>
Whole plant phenotyping of maize diversity lines reveals distinct outcomes to seedling water limitation

P78 **Kokulapalan Wimalanathan** <kokul@iastate.edu>
Bulked Segregant - genotyping-by-sequencing: Cost-effective and background independent genetic mapping of mutants and QTL

P79 **Kokulapalan Wimalanathan** <kokul@iastate.edu>
Maize - GO Annotation Methods Evaluation and Review (Maize-GAMER)

Biochemical and Molecular Genetics

P80 **Tes Posekany** <posekany@iastate.edu>
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<abrahammj@berkeley.edu>
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<yuguo_xiao@byu.edu>
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<jjs369@cornell.edu>
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<martinalexander@berkeley.edu>
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<gefei511@waksman.rutgers.edu>
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<edemesaa@cshl.edu>
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<dale.brunelle@und.edu>
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<debamalya1989@gmail.com>
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<jjs369@cornell.edu>
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<qwu@cshl.edu>
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<nichomiles@gmail.com>
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<sleiboff@berkeley.edu>
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<zhaoxial@iastate.edu>
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<steve@cau.edu.cn>
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<warmanma@oregonstate.edu>
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<jmendo01@unm.edu>
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<mli@danforthcenter.org>
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<hilde.nelissen@psb.vib-ugent.be>
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<xxzheng@sibs.ac.cn>
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<jwsgk9@mail.missouri.edu>
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<milst023@umn.edu>
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<lundec@berkeley.edu>
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P249 **Chunhui Xu** <chunhuixu@sdu.edu.cn>
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P252 **James Birchler** <birchlerj@missouri.edu>
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P254 **Wei Huang** <wilsonhuang23@cau.edu.cn>
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P256 **Morgan McCaw** <mem7b6@mail.missouri.edu>
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P261 **Savannah Savadel** <sds14d@my.fsu.edu>
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Education & Outreach

P265 **Robert Meeley** <bob.meeley@pioneer.com>
CRISPR-Cas advanced breeding to produce next generation waxy corn products

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A new dominant dwarfing mutant of maize exhibiting exquisite sensitivity to the genetic background

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Canalization in the phosphate-starvation response of a Mexican maize landrace native to acidic volcanic soils

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P287 **Julia Kleinmanns** <jakleinmanns@ucsd.edu>
Comparative analysis of GRN and eQTL modules in maize

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Complex genetic architecture of maize domestication traits as explained by interaction between teosinte branched 1 (tb1) and its genetic background

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Complex genetic architecture underlies maize tassel domestication

P290 **Candice Gardner** <candice.gardner@ars.usda.gov>
Composite selection mapping in three exotic maize populations: adaptation to the central U.S. Corn Belt

P291 **Jinyu Wang** <jinyuw@iastate.edu>
Connection between genome divergence patterns and DNA repair systems in crops

P292 **Brett Burdo** <burdo@wisc.edu>
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P297 **Lei Liu** <lliu@cshl.edu> — *Dissecting genetic architecture of maize domestication traits and predicting candidate genes using a teosinte-maize population*

P298 **Laura Morales** <lm596@cornell.edu> — *Dissecting the genetics and mechanisms of the maize-Fusarium verticillioides pathosystem in four NAM families*

P299 **Aaron Kusmec** <amkusmec@iastate.edu> — *Distinct genetic architectures for phenotype means and plasticities in Zea mays*

P300 **Srinivasa Chaluvadi** <src@uga.edu> — *Diversity of total and active nitrogen-fixing microbiome in the roots of selected grass species*

P301 **Qi Mu** <qmu@iastate.edu> — *Effect dynamics of qHT7.1 and Dw3, repulsion linked QTLs contributing to sorghum plant height heterosis*

P302 **Bridget McFarland** <bridgetm@iastate.edu> — *Evaluating kernel qualities following the integration of the brown midrib 3 mutation into soft endosperm maize lines*

P303 **Dnyaneshwar Kadam** <kadam013@umn.edu> — *Evaluation of nonparametric genomic selection models for predicting Single-Cross performance in maize*

P304 **Shangang Jia** <shangang.jia@gmail.com> — *Exome-seq based mapping tool to identify causal genes in maize kernel mutants*

P305 **Nick Ames** <amesx083@umn.edu> — *Exploiting genome by environment interaction in genomewide selection in maize*

P306 **James Chamness** <jchamness@gmail.com> — *Exploring the genetic basis of leaf cuticular evaporation rate in maize*

P307 **Alessandra York** <torno@wisc.edu> — *Fine-mapping a major maize domestication QTL for ear diameter*

P308 **Frank McFarland** <fmcfarland@wisc.edu> — *Fine-mapping of a QTL associated with somatic embryogenesis and plant regeneration in maize tissue culture*

P309 **Christy Gault** <cg449@cornell.edu> — *Freezing tolerance is associated with higher photosynthetic performance during chilling stress in Tripsacum, the sister genus of maize*

P310 **Greg Ziegler** <gziegler@danforthcenter.org> — *Gene by Environment interactions of the maize ionome: lessons from NAM and the G2F project*

P311 **Nina Chumak** <nina.chumak@botinst.uzh.ch> — *Generating clonal progeny in maize*

P312 **Anna Glowinski** <acs5fd@mail.missouri.edu> — *Genetic analysis of a unique synthetic population: the Zea Synthetic*

P313 **Ijeoma Akaogu** <iakaogu@wacci.edu.gh> — *Genetic analysis of early-maturing maize inbreds containing genes from Zea diploperennis under Striga-infested and drought environments*

P314 **Xiaoyue Zhang** <xzhng128@illinois.edu> — *Genetic analysis of host resistance to Setosphaeria turcica, the causal agent of northern corn leaf blight and sorghum leaf blight*

P315 **Yingni Xiao** <xyn_xyn@126.com> — *Genetic architecture of kernel composition in a maize natural population*

P316 **Jessica Bubert** <jbubert2@illinois.edu> — *Genetic characterization of harvest and broom quality traits in broomcorn*

P317 **Kathryn Michel** <kathrynhoemann@gmail.com> — *Genetic dissection of morphological and anatomical traits using multi-parent advanced generation intercross populations of maize*

P318 **Peter Balint-Kurti** <pjbalint@ncsu.edu> — *Genetic dissection of the maize MAMP response*

P319 **Mercy Kabahuma** <kabahuma@iastate.edu> — *Genetic dosage analysis of alleles conferring quantitative disease resistance to Setosphaeria turcica, the causal agent of northern corn leaf blight*

P320 **Matthew Dzievit** <mdzievit@iastate.edu> — *Genetic mapping and introgression of leaf angle QTL in maize*

P321 **Ruixiang Liu** <maize2008@hotmail.com> — *Genetic mapping of QTL for maize leaf width combining IF2 and RIL populations*

P322 **Megan Fenton** <fentonm@purdue.edu> — *Genome wide association analysis of Striga hermonthica resistance in a sorghum MAGIC population*

P323 **Di Wu** <dw524@cornell.edu> — *Genome-wide association study and genomic prediction models for mineral levels in maize grain*

P324 **Yingjie Xiao** <shanren0179@163.com> — *Genome-wide association study dissects the genomic treasures of elite maize inbred lines*

P325 **Masanori Yamasaki** <yamasakim@tiger.kobe-u.ac.jp> — *Genome-wide association study using whole-genome sequencing rapidly identifies new genes influencing agronomic traits in rice*

P326 **Thiago Marino** <tpmarino@ncsu.edu> — *Genomewide recurrent selection for Fusarium ear rot and Fumonisin resistance in maize*

P327 **Kelly Swarts** <kelly.swarts@tuebingen.mpg.de> — *Genomic estimation of complex traits in archaeological maize reveals ancient adaptation to temperate North America*

P328 **Zhi Li** <lixx5447@umn.edu> — *Genotype-by-environment interactions affecting heterosis in maize*

P329 **Lorena Rios-Acosta** <lrios@illinois.edu> — *Genotypic diversity in the responses of yield and yield components to elevated ozone of diverse inbred and hybrid maize*

P330 **Esperanza Shenstone** <shensto2@illinois.edu> — *Harnessing genomic variability to reduce stalk lodging in maize*

P331 **Alain Charcosset** <charcos@moulon.inra.fr> — *Heterosis in maize: intermated recombinant inbred lines and their immortalized F2 reveal (i) QTLs with dominant effects but no overdominance and (ii) a large contribution of epistasis for grain yield*

P332 **Rajanikanth Govindarajulu** <rajini28m@gmail.com> — *High density bin mapping in a sorghum RIL population [S. propinquum × S. bicolor (Tx7000)] for comparative analyses with foxtail millet and maize to determine the genetic architecture of tillering*

P333 **Jeffrey Ross-Ibarra** <rossibarra@ucdavis.edu> — *HiLo: Evolutionary genetics of highland adaptation in maize and teosinte*

P334 **Yan Zhou** <yzhou86@iastate.edu> — *Identification of genetic determinants of maize tassel structure*

P335 **Fei Lu** <fl262@cornell.edu> — *Identifying deleterious mutations in maize*

P336 **Darshi Banan** <banan2@illinois.edu> — *Influence of leaf rolling on canopy light environment and yield response to drought revealed by hemispherical imaging in Setaria*

P337 **Ravi Valluru** <rv285@cornell.edu> — *Integration of physiology and genetic load with genome wide prediction for drought tolerant sorghum*

P338 **Sarah Hill-Skinner** <shillski@iastate.edu>
Intra-specific variation for carbon sequestration potential in maize

P339 **Alina Ott** <aott@iastate.edu>
Intragenic meiotic recombination generates novel gene expression patterns

P340 **Nannan Liu** <935019928@qq.com>
Intraspecific variation of residual heterozygosity and its utility for quantitative genetics studies in maize

P341 **Mike White** <mrwhite4@wisc.edu>
Investigating kinship and heterotic relationships of expired maize plant variety protection lines

P342 **Heather Manching** <hcorn@udel.edu>
Investigating the genetics of selection response for flowering time in a multi-environment parallel selection experiment

P343 **Zhengbin Liu** <zliu@danforthcenter.org>
Key loci playing pleiotropy roles during maize domestication identified by QTL analysis of leaf-root-ionome in TeoNILs

P344 **Crystal A. Sorgini** <sorgini2@illinois.edu>
Mapping oxidative stress response QTL in B73 - Mo17 NILs

P345 **Jiaojiao Ren** <renjiaojiao789@sina.com>
Mapping QTL for spontaneous haploid genome doubling (SHGD) under selective genotyping

P346 **Chenxu Liu** <liuchenxusdau@126.com>
Marker assisted selection of in vivo maize haploid inducers suited for automatic haploid identification

P347 **Celeste Falcon** <cfalcon@wisc.edu>
Morphological characterization and genetic dissection of maize yield component traits collected by image analysis

P348 **Mark Holmes** <holme616@umn.edu>
Natural variation for food grade corn quality traits relevant to chip processing

P349 **Christine Diepenbrock** <chd45@cornell.edu>
Networks of genetic control for maize grain carotenoid levels in the US-NAM panel

P350 **Weiya Li** <liweiya@cau.edu.cn>
New insights on Cellulose Synthase-Like D1 controlling organ size in maize

P351 **Julian Cooper** <julianscottcooper@gmail.com>
Novel alleles for Goss' Wilt resistance to maize

P352 **Michael Anokye** <anomic17@gmail.com>
Phosphate nutrition during early growth of landrace maize (Zea mays L.) originating from the volcanic soils of the mexican highlands

P353 **Vlatko Galic** <vlatko.galic@poljinos.hr>
Photosynthesis and grain yield in the field environments managed for drought stress during flowering: QTL mapping for testcross performance in IBM population

P354 **Ying Ren** <renying900115@hotmail.com>
Progress in the development of Quality Protein Popcorn (QPP)

P355 **Raeann Goering** <rgoering01@hamline.edu>
QTL Analysis of Cold Tolerance in Maize

P356 **Lora Daskalska** <ldaskalska@wisc.edu>
QTL mapping for days to antithesis (DTA) and days to silk (DTS) in multiple maize-teosinte hybrid populations

P357 **Popi Septiani** <popi.septiani@santannapisa.it>
QTL Mapping for Fusarium ear rot resistance in the MAGIC maize population

P358 **Craig DeValk** <cdevalk@wisc.edu>
QTL mapping for tiller number in multiple maize-teosinte hybrid populations

P359 **Jason Wallace** <jason.wallace@uga.edu>
Quantitative analysis of the maize leaf microbiome

P360 **Jonathan Renk** <jrenk@wisc.edu>
Relationship between maize (Zea mays L.) whole plant silage quality and ear morphological characteristics per whole plot basis

P361 **Angela Chen** <chen398@illinois.edu>
Simulation results suggest stepwise selection algorithm picks up epistatic signals in the US maize nested association mapping panel

P362 **Sofia P. Brandariz** <bran0795@umn.edu>
Small ad hoc versus large general training populations for genomewide selection in maize biparental crosses

P363 **Addie Thompson** <thomp464@purdue.edu>
Stability and tradeoff of allelic effects in drought-treated elite temperate and tropical maize hybrids

P364 **Jeremy Pardo** <jdp267@cornell.edu>
Stomatal behavior and transcriptional response of 27 maize genotypes to drought

P365 **Madeline McMullen** <mmcm@iastate.edu>
The challenge of poor penetrance by pangloss1 while breeding maize lines to test its impacts on yield

P366 **Judith Kolkman** <jmkolkman@gmail.com>
The disease profile of the brown midrib mutants in maize

P367 **Joseph Gage** <jgage2@wisc.edu>
The effect of artificial selection on phenotypic plasticity in maize

P368 **Nicholas Heller** <njhelle2@illinois.edu>
The FLOURY2 zein-RFP transgene – a phenotype to investigate multiple modes of regulation

P369 **Xiaowei Li** <lixiaowei810@126.com>
The genome-wide association study dissects the genetic architecture of embryo size in maize

P370 **Donald Auger** <donald.auger@sdstate.edu>
The high-amylose trait of GEMS-0067 is due to an allele of starch branching enzyme 1 that appears to be literally wild-type

P371 **Merritt Burch** <merritt.b.burch@sdstate.edu>
The hunt for modifiers of the Tcb-1 locus

P372 **Brian Rice** <brice6@illinois.edu>
The influence of peak GWAS signals on genomic prediction accuracy

P373 **Max Feldman** <mfeldman@danforthcenter.org>
Understanding plant functional morphology, water use and cellular composition using structured genetic populations of Setaria viridis

P374 **Cinta Romay** <mcr72@cornell.edu>
Understanding the regions that control grain yield with Ames and NAM hybrids

P375 **Keith Duncan** <kduncan@danforthcenter.org>
Using X-ray computed tomography to quantify Micro- and Macro-Morphology of maize and other plants

P376 **Lais Bastos Martins** <lbastos@ncsu.edu>
Validation of Multiple Disease Resistance loci in Maze using families derived from segment substitution lines

P377 **Garrett Janzen** <gjanzen@iastate.edu>
Verification of highland/lowland adaptation in maize landraces by reciprocal transplantation

P378 **Mengqiao Han** <mhan16@illinois.edu>
Water use efficiency in hybrid maize

Transposons & Epigenetics

P379 **William Ricci** <william.ricci@uga.edu>
A method for enrichment of maize stem cells and leaf primordia

P380 **Mithu Chatterjee** <cmithu@waksman.rutgers.edu>
A sequenced-indexed reverse genetics resource for maize

P381 **Thelma Madzima** <madzima@uw.edu>
Abiotic stress induced nucleosome occupancy profiling in maize

P382 **Jaclyn Noshay** <nosha003@umn.edu>
Application of ATAC-seq to monitor variation in open chromatin among maize tissues and genotypes

P383 **Allison McClish** <mcclish.23@osu.edu>
Cell-autonomous action of RNA polymerase IV maintains the epigenetic repression of a paramutant pl1 allele

P384 **Jin Cui** <juc326@psu.edu>
Characterization of a candidate gene for Ufo1

P385 **Wei Xue** <wxue22@wisc.edu>
Characterization of transgenerational epigenetic inheritance of sickly syndrome in a specific maize-teosinte backcross population

P386 **Jonathan Gent** <gent@uga.edu>
Chromatin modifications of repetitive DNA

P387 **Sharu Paul Sharma** <sharu@iastate.edu>
Chromosomal inversions caused by alternative transpositions in maize

P388 **Na Wang** <na.wang25@uga.edu>
Comparison of centromere size and location in different maize accessions and their hybrids

P389 **Weijia Su** <weijia@iastate.edu>
Composite Insertions (CIs) and the evolutionary impact of Reversed-Ends Transposition (RET) in maize

P390 **Miaoyun Xu** <xumiaoyun@caas.cn>
Comprehensive analysis of lncRNA, mRNA, circRNA and miRNA expression uncovers a complex regulatory network that affects maize seed development

P391 **Dafang Wang** <wang2630@purdue.edu>
Dualism of Muk silencing: the transition between the transcriptional gene silencing and translational/post-translational inhibition during the Muk-induced silencing at various maize developmental stages

P392 **Sarah Anderson** <sna@umn.edu>
Dynamic transposable element expression across development and stress in maize

P393 **Alice Pieri** <alice.pieri@santannapisa.it>
Exploring long noncoding RNAs in wheat wild relatives

P394 **Dexuan Meng** <xuan_0515@126.com>
Gene expression pattern of early maize embryo

P395 **James Whelan** <jwhelan01@hamline.edu>
Genotyping with UniformMu insertion elements

P396 **Kazuhiro Kikuchi** <kkikuchi@danforthcenter.org>
High throughput identification of Ds insertion sites in maize using NGS

P397 **Dhanushya Ramachandran** <dramacha@mix.wvu.edu>
LTR-Retrotransposon dynamics in the Andropogoneae

P398 **Shujun Ou** <oushujun@msu.edu>
LTR_retriever: a highly sensitive and accurate program for identification of LTR retrotransposons

P399 **Myron Neuffer** <gneuffer@gmail.com>
Mechanical chemical and CRISPR mutagenesis in perspective

P400 **Michelle Stitzer** <mcstitzer@ucdavis.edu>
Multiple maize reference genomes allow insight into intergenic transposable element evolution

P401 **Maike Stam** <m.e.stam@uva.nl>
Paramutation at the b1 locus is associated with RdDM activity at the paramutable B-I allele

P402 **Fang Bai** <fbai001@ufl.edu>
Parent-of-origin effect rough endosperm mutants alter cellular development of the endosperm in maize

P403 **Jinliang Yang** <jolyang@ucdavis.edu>
Population genetic modeling of methylation variation in a natural teosinte population

P404 **Bosen Zhang** <bszhang@illinois.edu>
Regulatory network analysis of maize small RNAs identifies modules associated with productivity traits

P405 **Meixia Zhao** <zhao185@purdue.edu>
RNA-directed DNA methylation components affect Mutator transposon insertion preferences, spontaneous silencing and double strand break repair

P406 **Jay Hollick** <hollick.3@osu.edu>
Stability of a paramutant pl1 allele is affected by a heritable cytoplasmic component

P407 **Xinyan Zhang** <zhan2168@purdue.edu>	*Transcriptional silencing of helitron-embedded miRNA target mimicry by RNA-directed DNA methylation (RdDM) and histone methylation*
P408 **Jin Cui** <cuijinjincui4@gmail.com>	*Transcriptome analysis reveal candidate genes for Ufo1*
P409 **Emily McCormic** <mccormic.11@osu.edu>	*Two mutations define the required to maintain repression10 locus affecting locus-specific paramutation*
P410 **Chunlei Wang** <wangchunlei3405@163.com>	*ZmEMFL1 is involved in regulating imprinted genes through H3K27me3 in Maize*
P411 **Zi Shi** <shizi_baafs@126.com>	*Effect of saline stress on the physiology and growth of maize hybrids and their related inbred lines*
P412 **Zi Shi** <shizi_baafs@126.com>	*Comparative proteomic analysis of two maize inbred lines upon long-term saline treatment*
P413 **Zi Shi** <shizi_baafs@126.com>	*Mapping a major QTL for salt tolerance of mature maize plant in field based on SNP markers*
P414 **Ramesh Dhakal** <rdhakal06@gmail.com>	*QTL mapping coupled with expression profiling identifies potential genes for aflatoxin resistance in corn*
P415 **Carolyn Lawrence-Dill** **<**triffid@iastate.edu**>**	*From Medicine to Plant Sciences: the Promise of Computing on Phenotypic Descriptions for Predictive Phenomics*
P416 **Carolyn Lawrence-Dill** **<**triffid@iastate.edu**>**	*Crowdsourcing for Ground Truth: Using the Amazon Mechanical Turk to Collect Data for Image Analysis using Machine-Learning*

Plenary Talk Abstracts

Plenary 1 **Thursday, March 9 7:15 PM**

Shedding light on the dark spaces of the maize genome

(presenter: Nathan Springer <springer@umn.edu>)
Full Author List: Springer, Nathan[1]
[1] Department of Plant and Microbial Biology; University of Minnesota; St. Paul, MN, 55108, USA

Less than 5% of the bases in the maize genome are located within genes (exons, introns and untranslated regions). The remaining portion of the genome includes repetitive sequences, such as transposable elements (TEs), as well as regulatory information. The maize genome is a rich resource for studying the regulation of transposons and their influence on nearby genes. Improved genome assemblies coupled with new technologies are providing avenues to interrogate these complex genomic regions. Profiles of DNA methylation and other chromatin marks have revealed variation in the chromatin modifications associated with particular TE families. Mutations that influence genomic DNA methylation in maize affect the regulation of a subset of TE families, highlighting the importance of epigenetic regulation of TE expression. There is evidence that some TE families influence the chromatin of nearby low-copy regions. Given the high rate of TE polymorphisms among maize haplotypes this creates the potential for genetically induced variation in DNA methylation through TEs that can influence gene expression. While TEs are under-represented in the transcriptome (relative to their proportion in the genome) they are not all silenced. A subset of TEs are expressed and often exhibit dynamic regulation in different tissues or in response to environmental conditions. In some cases, these TEs appear to provide sources of novel regulatory variation that can provide gene expression responsiveness to environmental cues and could allow for selection of advantageous gene expression patterns. A more detailed understanding of the role of chromatin and TEs in intergenic space could provide avenues to target important regulatory features of the maize genome and epigenome for crop improvement.

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Sacrificing for security? Trade-offs related to disease resistance in plants

(presenter: Rebecca J. Nelson <rjn7@cornell.edu>)
Full Author List: Nelson, Rebecca J[1]; Kolkman, Judith M[1]; Morales, Laura[1]; Weisner-Hanks, Tyr[1]; Jamann, Tiffany M[2]; Poland, Jesse A[3]; Mideros, Santiago X[2]; Wisser, Randall J[4]; Balint-Kurti, Peter J[5]
[1] Cornell University; Ithaca, NY
[2] University of Illinois; Champaign-Urbana, IL
[3] Kansas State University, Manhattan, KS
[4] University of Delaware, Newark, DE
[5] North Carolina State University, Raleigh, NC

Plants defend themselves from pathogens using a variety of mechanisms, and each approach to defense is associated with potential downsides. Conversely, crop improvement can influence disease susceptibility as other breeding goals are pursued. There is a fundamental trade-off associated with effector-triggered immunity, plants' strongest and most specific type of resistance; while it can be very effective, it is often rapidly overcome as pathogens evolve to evade recognition and/or suppress defenses. Other costs of qualitative resistance may apply, as illustrated by findings in Arabidopsis and other systems. Quantitative resistance is believed to be more durable, but may also be associated with undesirable traits due to linkage and/or pleiotropy. As the genetic architecture of quantitative resistance becomes better understood, the linkage relationships of resistance loci and those affecting other traits can be assessed and co-localizing traits can be identified. The genetic basis for quantitative resistance is just beginning to be revealed. There is emerging evidence that genes involved in quantitative recognition include those with roles in recognition and signal transduction and response; plant development and structure (macroscopic and microscopic); biochemistry; hormones; and restriction of pathogen nutrition. Observed and potential trade-offs associated with some of these mechanisms will be discussed in maize and other systems. The impacts of ear traits on the pathogenesis of mycotoxigenic fungi is of particular interest in maize in tropical contexts. Breeding for high yield may exacerbate the toxicity of the African food system.

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

Plant Immunity – The Signaling Network and the Role of a Family of Calmodulin-Binding Proteins

(presenter: Jane Glazebrook <jglazebr@umn.edu>)
Full Author List: Glazebrook, Jane[1]; Lu, You[1]; Liu, Xiaotong[1,2]; Bethke, Gerit[1]; Zhou, Man[1]; Katagiri, Fumiaki[1]
[1] Department of Plant and Microbial Biology; University of Minnesota; St. Paul, MN, 55108, USA
[2] Graduate Program in Bioinformatics and Computational Biology; University of Minnesota; St. Paul, MN 55108, USA

Plants adjust their metabolism and growth to a fluctuating environment. I will focus on how they adjust to a changing supply of carbon. I will start by considering how carbon supply and growth are coordinated during vegetative growth. During the day, plants carry out photosynthesis. Part of the fixed carbon is temporarily stored as starch, and remobilized to support metabolism and growth at night. It has been known since the 1980's that starch breakdown is regulated such it is almost but not totally exhausted at the end of the night. This optimizes growth while minimizing the risk of periods of carbon starvation. I will discuss how we have used a range of 'omics approaches to characterize this response and have identified a key role for the biological clock in regulating the rate of starch breakdown and the timing of growth during diurnal cycles. While doing this I will introduce new methods for measuring expansion growth, and for quantifying the rates of protein and cell wall synthesis. I will then discuss the role of the sugar-signaling molecule trehalose-6-phosphate in adjusting metabolism and allocation to the carbon supply, including new results on it role in regulating carbon-nitrogen interactions. In the last part of the talk I will consider how carbon-signaling regulates key developmental transition like flowering, branching and seed set, which set up a future demand for carbon. The talk will focus on studies with Arabidopsis but I will try to draw parallels with maize.

Funding acknowledgement: National Science Foundation (NSF, IOS 1353854), F.K. (MCB-1518058)

Regulation of endosperm development: Insights and surprises

(submitted by Philip Becraft <becraft@iastate.edu>)
Full Author List: Becraft, Philip W.[1]; Neelakandan, Anjanasree[1]; Wu, Hao[1]; Gontarek, Bryan[1]
[1] Iowa State University, Ames, IA 50011

Cereal endosperm functions to support embryogenesis during grain development and seedling development during germination. It is also an extremely important commodity. Endosperm development entails a period of coenocytic development followed by cellularization progressing from the periphery, centripetally toward the center of the endosperm. Periods of rapid cell proliferation, cell differentiation, growth, storage product deposition and seed maturation follow. Maize endosperm contains 7 described cell types, each with specialized structures, functions and gene expression patterns. Classical molecular genetic studies have provided insights on some of the factors required for endosperm development. Empty pericarp and severe defective kernel mutants identify essential genes and a high proportion of such genes are involved in organellar functions such as mitochondrial RNA editing. Of the genes identified that regulate the differentiation and function of key cell types including starchy endosperm, transfer cells and aleurone, most of have pleiotropic effects. All the described mutants that disrupt aleurone differentiation also show phenotypic effects in the starchy endosperm. Recent transcriptomic analyses have explained the bases for much of this. For example, NKD transcription factors required for aleurone differentiation also regulate genes involved in carbohydrate metabolism, storage protein deposition and carotenoid biosynthesis. They also regulate expression of other key transcription factors such as O2. Ongoing analyses are revealing the modular nature of the gene regulatory networks that underlie endosperm development.

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

McClintock Prize Abstract

McClintock Prize **Friday, March 10 7:30 PM**

How polyploidy promotes evolvability, and how that's not enough to explain the distribution of paleopolyploidies during plant evolution

(presenter: Michael Freeling <freeling@berkeley.edu>)
Author list: Freeling, Michael[1];
[1] Plant and Microbial Biology Department, University of California, Berkeley CA 94720

The distribution of inferred paleopolyploidies in the inferred phylogenetic tree of vascular plants is difficult to understand. It not just that **every** existing angiosperm is the result of repeated polyploidies, but that over three polyploidies per lineage are the rule, not the exception, and we can only see back into genomes for, say, 120 MY. I will turn the question "What makes polyploids so successful?" upside down and ask "What makes polyploids so hard to kill?"?

As to evolvability, most suggested advantages of polyploidy-- following E.B. Lewis for genes and S. Ohno for genomes-- involve the relaxation of selection, and the eventual emergence of novelty. Fractionation of genes and cis-regulatory sites and subfunctionalization happen because selection is relaxed. The traditional view: novelty emerges via recombination among divergent alleles and step-by-small-step selection (The Modern Synthesis), without the need for "spectacular new mutations". After reviewing what is known about the mechanical consequences of polyploidy – including the phenomenon of genome dominance in ancient allopolyploids, and its possible epigenetic mechanism via *cis* position-effects of silenced transposons near genes, and how these mechanisms alter gene content over time-- I will show that any advantages of polyploidy are expected to be out-weighed by the disadvantages of the new polyploid.

Stebbins and, more recently, Soltis, D.E., Visger, C.J., and Soltis, P.S. (2014. The polyploidy revolution then...and now: Stebbins revisited. Am J Bot 101, 1057-1078) came to the conclusion that recently polyploid plants today are evolutionary dead ends. The general idea that macroevolution (origin of genera…) is going on today, as assumed by the Modern Synthesis, is not fully accepted (read Goldschmidt, 1953, Am Sci 40: 84-98). New polyploids happen in ways that avoid allelic diversity and new polyploids have lowered fitness due to problems at meiosis. So, how could they possibly be "hard to kill" when they seem to die-off quickly all on their own merits? I will suggest that the distribution of polyploidies in plant lineages is a spandrel of asexual reproduction with occasional sex (at the printers, The Plant cell). While the polyploids are hiding out from extinction-making ionizing radiations underground or underwater for millions of years—doing nicely vegetatively, like bamboo clonal forests or *Elodea* masses—mutational diploidizations occur and, once in a great while, a sexual flower emerges and the advantage of sex can be realized once again in the lineage. This mutationist "spandrel" explanation, and similar ideas, are wrongly excluded from The Modern Synthesis.
Funded by NSF, PGRP.

Short Talk Abstracts

SESSION 2 – THE MAIZE GENOMES
Chair: Gernot Presting Friday, March 10. 8:00 AM – 9:55 AM

T1

A novel maize kinesin causes neocentromere activity and meiotic drive, altering inheritance patterns across the genome

(submitted by Kelly Dawe <kdawe@uga.edu>)
Full Author List: Dawe, R. Kelly[1]
[1] University of Georgia, Athens GA, 30602

Mendelian inheritance is a foundational principle of genetics, yet there are numerous meiotic drive systems that alter genetic segregation to favor their own transmission. Maize abnormal chromosome 10 (Ab10) encodes a classic example of "true meiotic drive" that converts heterochromatic regions called knobs into motile neocentromeres that are preferentially transmitted to egg cells. We have identified a nine-gene family called the Kinesin driver (Kindr) complex that is unique to Ab10 and responsible for this phenotype. Two mutants of meiotic drive proved to be spontaneous kindr epimutants that show increases in DNA methylation and changes in small RNA targeting across the entire gene family. RNAi of Kindr induced a third stable epimutant, inactivating the gene complex and abolishing meiotic drive. Kindr evolved ~12 mya from a minus-end directed kinesin-14A gene and rapidly expanded in copy number by unequal crossing over and gene conversion. The Kindr gene family has been one of the most powerful forces in the evolution of Zea, driving the accumulation of hundreds of megabases of knob repeats and altering the segregation patterns of thousands of genes linked to knobs on all 20 chromosome arms.

Funding acknowledgement: National Science Foundation (NSF)

T2

Evolutionary genomics of European maize and its American counterparts

(submitted by Maud Tenaillon <maud.tenaillon@inra.fr>)
Full Author List: Brandenburg, Jean-Tristan[1]; Marie-Huard, Tristan[1]; Rigaill, Guillem[2]; Hearne, Sarah[3]; Corti, Hélène[1]; Joets, Johann[1]; Vitte, Clémentine[1]; Charcosset, Alain[1]; Nicolas, Stéphane[1]; Tenaillon, Maud[1]
[1] Génétique Quantitative et Evolution – Le Moulon; INRA, Univ Paris-Sud, CNRS, AgroParisTech, Univ Paris-Saclay; Ferme du Moulon; 91190; Gif-sur-Yvette
[2] Institute of Plant Sciences Paris-Saclay; CNRS, INRA, Université Paris-Sud, Université d'Evry, Université Paris-Diderot, Sorbonne Paris-Cité; Gif-sur-Yvette; F 91190
[3] CIMMYT; Texcoco; Edo de Mexico; 56237 Mexico

We sequenced 67 genomes with an average sequencing depth of 18x to document routes of introduction, admixture and selective history of European maize and its American counterparts. To avoid the confounding effects of recent breeding, we targeted germplasm (lines) directly derived from landraces. Among our lines, we discovered 22,294,769 SNPs and between 0.9% to 4.1% residual heterozygosity. We developed a segmentation method to identify 6,977 segments of unexpectedly high rate of heterozygosity, pointing to either underlying structural variants or genes potentially involved in inbreeding depression. Genetic structuring and inferences of historical splits revealed 5 genetic groups and two 2 independent European introductions, with modest bottleneck signatures. Our results further revealed admixtures between distinct sources that have contributed to the establishment of 3 groups at intermediate latitudes in North America and Europe. We combined differentiation- and diversity-based statistics to identify both genes and gene networks displaying strong signals of selection. These include genes/gene networks involved in flowering time, drought and cold tolerance, plant defense, and starch properties. Overall, our results provide novel insights into the evolutionary history of European maize and highlight a major role of admixture in environmental adaptation.

Funding acknowledgement: French National Research Agency (Amaizing, ANR-10-BTBR-03). France Agrimer. LabEx BASC (ANR-11-LABX-0034).

T3

Genetic diversity and genomic constitution of ancient Tehuacan maize dated 5300 to 5000 years before present

(submitted by Miguel Vallebueno-Estrada <miguel.vallebueno@cinvestav.mx>)
Full Author List: Vallebueno-Estrada, Miguel A[1 2]; Rodríguez-Arévalo, Isaac[1]; García-Morales, Sara[1 2]; Martínez González, Javier[3]; García-Cook, Angel[3]; Montiel, Rafael[2]; Vielle-Calzada, Jean-Philippe[1]
[1] Grupo de Desarrollo Reproductivo y Apomixis, Unidad de Genómica Avanzada, Laboratorio Nacional de Genómica para la Biodiversidad, Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional Irapuato, 36821 Guanajuato, Mexico.
[2] Grupo de Interacción Núcleo-Mitocondrial y Paleogenómica, Unidad de Genómica Avanzada, Laboratorio Nacional de Genómica para la Biodiversidad, Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional Irapuato, 36821 Guanajuato, Mexico.
[3] Instituto Nacional de Antropología e Historia, 06100 Mexico DF, Mexico

A long history of archaeobotanical records indicates that the Tehuacan Valley in México was an important center of early Mesoamerican agriculture, providing evidence of the complexity of maize domestication over the last 5,000 years. Owing to expeditions conducted by MacNeish and his team in the early 1960s, we conducted a new exploration of several rockshelters located in different regions of the Tehuacan valley, uncovering more than 100 nonmanipulated maize specimens dating from 5,300 to 1,000 years before present (BP), and allowing genetic comparisons that incorporate both the temporal and geographical scale to the perspective of ancient maize evolution. Our initial studies show that the earliest maize from San Marcos was a partial domesticate diverging from the landraces and containing ancestral allelic variants that are absent from extant maize populations across the genome, particularly at several loci important for domestication. The genomic comparison of three temporally convergent 5300-5000 BP samples indicated that they were unusually homozygous and genetically similar, suggesting the earliest maize from San Marcos was already inbred. The de novo assembly of their genome revealed structural re-arrangements as well as unique insertion-deletion and gene variants that are likely to be absent from extant populations. We hypothesize that this structural variation could be related to environmental adaptations or to the size and structure of ancient maize populations. Our studies open new perspectives for discovering past genetic variability that could be useful for present or future agricultural conditions.

Funding acknowledgement: CONACYT-MEXICO

T4

MATRILINEAL, a sperm-specific phospholipase, triggers maize haploid induction

(submitted by Tim Kelliher <tim.kelliher@syngenta.com>)
Full Author List: Kelliher, Timothy[1]; Starr, Dakota[1]; Richbourg, Lee[1]; Chintamanani, Satya[2]; Delzer, Brent[3]; Nuccio, Michael L.[1]; Green, Julie[1]; Chen, Zhongying[1]; McCuiston, Jamie[1]; Wang, Wenling[1]; Liebler, Tara[1]; Bullock, Paul[2]; Martin, Barry[4]
[1] Seeds Research, Syngenta Crop Protection; 9 Davis Dr., Research Triangle Park, North Carolina, USA 27709
[2] Syngenta Seeds, Syngenta Crop Protection; Slater, Iowa, USA 50244
[3] Syngenta Seeds, Syngenta Crop Protection; Janesville, Wisconsin, USA 53546
[4] Present address: CiBO Technologies; Cambridge, Massachusetts, USA 02141

Sexual reproduction in flowering plants involves double fertilization, the union of two sperm from pollen with two sex cells in the female embryo sac. Modern plant breeders increasingly seek to circumvent this process to produce doubled haploid individuals, which derive from the chromosome-doubled cells of the haploid gametophyte. Doubled haploid production fixes recombinant haploid genomes in inbred lines, shaving years off the breeding process. Costly, genotype-dependent tissue culture methods are used in many crops, while seed-based *in vivo* doubled haploid systems are rare in nature and difficult to manage in breeding programmes. The maize hybrid seed business, however, is supported by industrial doubled haploid pipelines using intraspecific crosses to *in vivo* haploid inducer males derived from Stock 6, first reported in 1959, followed by colchicine treatment. Despite decades of use, the mode of action remains controversial. Here we establish, through fine mapping, genome sequencing, genetic complementation, and gene editing, that haploid induction in maize is triggered by a frame-shift mutation in *MATRILINEAL* (*MTL*), a pollen-specific phospholipase, and that novel edits in *MTL* lead to a 6.7% haploid induction rate. Wild-type MTL protein localizes exclusively to sperm cytoplasm, and pollen RNA-seq profiling identifies a suite of pollen-specific genes overexpressed during haploid induction, some of which may mediate the formation of haploid seed. These findings highlight the importance of male gamete cytoplasmic components to reproductive success and male genome transmittance. Given the conservation of *MTL* in the cereals, this discovery may enable the development of *in vivo* haploid induction systems to accelerate breeding in crop plants.

Funding acknowledgement: Internal Funding

T5

Understanding maize development through the Liguless narrow mutant and its phenotypic modifier: Sympathy for the ligule

(submitted by Alyssa Anderson <alyssa.amy@berkeley.edu>)
Full Author List: Anderson, Alyssa[1]; St. Aubin, Brian[2]; Shen, Zhouxin[3]; Abraham Juarez, Jazmin[1]; Briggs, Steve[3]; Hake, Sarah[1]
[1] Department of Plant and Microbial Biology, UC Berkeley, Berkeley, Ca, 94704
[2] Department of Plant Biology, Michigan State University, East Lansing, MI 48824
[3] Cell and Developmental Biology, UC San Diego, La Jolla, CA 92093

The maize mutant Liguleless narrow (Lgn-R) and its phenotypic modifier Sympathy for the ligule (Sol) provide an opportunity in which to study genotype, environment and development. Lgn codes for a kinase that, when mutated, has pleiotropic developmental defects such as decreased plant height and leaf width, and irregular ligule formation (Moon et al 2013). A modifier of this gene, Sol, was identified in a QTL analysis (Buescher et al 2014) and has now been cloned. Sol encodes a protein of unknown function with two zinc finger domains. Intriguingly only the version of Sol found in certain inbred lines, like Mo17, is capable of producing a rescued phenotype. An alternate version of Sol, found in inbreds such as B73, cannot rescue Lgn-R plants. Evidence indicates that the causal difference between the two versions of Sol is altered expression levels, possibly caused by changes in cis regulatory sites. Environmental influence also plays a role in the interaction between Lgn-R and Sol. At 23◦C Lgn-R plants in B73 show the mutant phenotype, while these same plants die at 33◦C. Lgn-R plants in a Mo17 background look almost WT at 23◦C but have a clear mutant phenotype at 33◦C. Phosphoproteome and RNAseq data indicate that the two genes either interact with or are part of the salicylic acid (SA) network. Further investigations into these two genes should reveal the mechanisms behind these drastic phenotypic differences and further illuminate their place in the regulatory networks of Zea mays.

Funding acknowledgement: National Science Foundation (NSF), UC Berkeley

T6

Natural variation in meristem size regulation: a naturally occurring hypomorphic allele of *thick tassel dwarf1* modifies the phenotype of *fasciated ear2* and increases kernel row number in maize

(submitted by Hannes Claeys <hclaeys@cshl.edu>)
Full Author List: Claeys, Hannes[1]; Vi, Son Lang[1]; Wu, Qingyu[1]; Bommert, Peter[1]; Dilkes, Brian[2]; Jackson, David[1]
[1] Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724, USA
[2] Purdue University, West Lafayette, IN 47907, USA

It has long been known that the genetic background affects the phenotype of mutations; however, it has been challenging to identify causative modifier loci. We used this phenomenon to better understand natural variation in meristem size regulation. The phenotype of *fasciated ear2* (*fea2*) null alleles, which causes hyperproliferation in inflorescence meristems leading to misshapen ears and tassels, is much stronger in the NC350 background compared to B73. We showed that this is due to a single major-effect modifier on chromosome 5, which we mapped to a region containing *THICK TASSEL DWARF1* (*TD1*). $TD1^{NC350}$ has no major changes in the coding region compared to the B73 reference, but exhibits a 2.5-fold reduction in expression levels. Interestingly, this naturally occurring weak allele of *TD1* causes an increase in kernel row number without affecting ear morphology, and could thus be useful in breeding programs.

Furthermore, we observed that about 14% of B73-NC350 recombinant inbred lines are fasciated, and identified loci different from *TD1* responsible for this fasciation. This further suggests that the NC350 genome contains loci that in isolation can lead to fasciation, but that these are normally balanced to ensure normal development. In order to investigate this compensatory mechanism that ensures that NC350 ears develop normally, we performed transcriptome analysis to look at the sequence and allele-specific expression levels of genes involved in meristem size regulation. These results provide a mechanistic insight in natural variation of meristem size regulation, a process that is crucial for proper development and has important agricultural consequences.

Funding acknowledgement: National Science Foundation (NSF), EMBO, DuPont-Pioneer

T7

Cloning of *Vgt3*, a major QTL for flowering time in maize

(submitted by Silvio Salvi <silvio.salvi@unibo.it>)
Full Author List: Emanuelli, Francesco[1]; Soriano, Jose Miguel[1]; Zamariola, Linda[1]; Giuliani, Silvia[1]; Bovina, Riccardo[1]; Ormanbekova, Danara[1]; Koumproglou, Rachil[2]; Burdo, Brett[3]; Rouster, Jacques[4]; Wyatt, Paul[4]; Tuberosa, Roberto[1]; Jahrmann, Torben[2 5]; Kaeppler, Shawn[3]; Praud, Sebastien[4]; Salvi, Silvio[1]
[1] DipSA - University of Bologna, Bologna, Italy
[2] Semillas Fito, Cabrera de Mar, Spain
[3] Department of Agronomy and DOE Great Lakes Bioenergy Research Center, University of Wisconsin - Madison, Madison, WI
[4] LIMAGRAIN, CHAPPES, France

Flowering time is a complex trait important for crop adaptation to local environments and an essential breeding target to face the challenge of global climate change. A major quantitative trait locus (QTL) for flowering time and number of nodes (ND), qVgt3.05 (Vgt3), was previously identified on chromosome 3, bin 3.05, in a maize introgression library (IL) derived from the cross B73 x Gaspé Flint (recipient and donor genotypes, respectively. Salvi et al. 2011). In order to clone Vgt3, B73 was crossed with its early isogenic line 39-1-2-33 which carries a 17-cM Gaspé Flint introgression on bin 3.05. Using this cross, Vgt3 showed an addictive effect of 1.4 nodes, explained 56.6% of the phenotypic variance and was mapped within 0.3 cM. For positional cloning, a total of 7,500 F2 plants were phenotyped and genotyped with SNPs and SSR markers flanking the QTL interval. One-hundred recombinants lines were derived and the QTL was further narrowed the target genomic region to a 380-kb interval. A MADS-box gene with no coding sequence variation between the two alleles was found in the physical interval. However, the MADS-box gene RNA expression profile and transgenics testing confirmed its effect on flowering time. We are currently searching for the Vgt3 causative regulatory region by studying chromosome structural variation between the B73 and Gaspé Flint alleles.

T8

Natural variation for Vitamin E content is controlled by a novel chlorophyll cycle in maize grain

(submitted by Dean DellaPenna <dellapen@msu.edu>)
Full Author List: DellaPenna, Dean[1]; Diepenbrock, Christine H[2]; Kandianis, Catherine B.[1 2 7]; Lipka, Alexander E.[3 8]; Magallanes-Lundback, Maria[1]; Vaillancourt, Brieanne[4]; Góngora-Castillo, Elsa[4]; Wallace, Jason G.[3 9]; Cepela, Jason[4]; Mesberg, Alex[1]; Bradbury, Peter J.[3 5]; Ilut, Daniel C.[2]; Mateos-Hernandez, Maria Mateos-Hernandez6[6 10]; Owens, Brenda F. Owens6[6]; Tiede, Tyler[6 11]; Buckler, Edward S.[2 3 5]; Buell, C. Robin[4]; Rocheford, Torbert[6]; Gore, Michael A.[2]
[1] Michigan State University, Department of Biochemistry and Molecular Biology, East Lansing, MI 48824
[2] Cornell University, Plant Breeding and Genetics Section, School of Integrative Plant Science, Ithaca, NY 14853
[3] Cornell University, Institute for Genomic Diversity, Ithaca, NY 14853
[4] Michigan State University, Department of Plant Biology, East Lansing, MI 48824
[5] United States Department of Agriculture-Agricultural Research Service (ARS), Robert W. Holley Center for Agriculture and Health, Ithaca, NY 14853
[6] Purdue University, Department of Agronomy, West Lafayette, IN 47907
[7] Bionutrient Food Association, North Brookfield, MA 01535
[8] University of Illinois at Urbana-Champaign, Department of Crop Sciences, Urbana, IL 61801
[9] University of Georgia, Department of Crop & Soil Sciences, Athens, GA 30602
[10] Monsanto Company, Stonington, IL, 62567
[11] University of Minnesota, Department of Agronomy and Plant Genetics, St. Paul, MN 55108

Tocopherols, tocotrienols, and plastochromanols, collectively called tocochromanols, are lipid-soluble antioxidants synthesized and accumulated in all plant tissues, where they have diverse functions critical to plant fitness. Dietary intake of tocochromanols, primarily derived from oilseed crops, provides Vitamin E and other beneficial health impacts. Tocopherol synthesis is fully elucidated in Arabidopsis and the corresponding orthologs are encoded by 80 genes (*a priori candidate genes*) in maize. To understand the genetic control of tocochromanols in staple crops we conducted a joint-linkage analysis and genome-wide association study in the maize nested association mapping (NAM) population. Of 52 tocochromanol quantitative trait loci (QTL) identified, 15 were resolved to their underlying causal gene and explain >65% of heritable variation for individual tocochromanol traits. Eight causal loci are members of the *a priori* candidate gene list and seven are *novel loci* encoding diverse activities not previously shown to affect tocochromanols in any plant system. That the two largest-effect tocopherol QTL were *novel loci* encoding protochlorophyllide reductases was surprising given that maize grain is a non-green, non-photosynthetic tissue. However, analysis of developing embryos from NAM parent lines showed specific chlorophyll metabolites that, while present at 500-10,000-fold lower levels than tocopherols, are highly correlated with total tocopherol synthesis. The combined genetic associations, metabolite analyses and embryo gene expression profiles indicate these chlorophyll metabolites participate in a new metabolic cycle that generates the large amount of phytol needed for tocopherol synthesis in maize grain. The 15 causal genes identified in this study, including seven *novel loci*, establish a comprehensive foundation for the genetic improvement of tocochromanol and vitamin E content in maize and most other major cereal crops whose grain, like maize, are also non-photosynthetic.

Funding acknowledgement: National Science Foundation (NSF)

T9

Return to near-normal starch accumulation: the unexpected contribution of pullulanase in a *su1-ref* background.

(submitted by Stacie Shuler <sshuler@wisc.edu>)
Full Author List: Shuler, Stacie L.[1]; Boehlein, Susan D.[2]; Hannah, L. Curtis[2]; Hennen-Bierwagen, Tracie A.[3]; Myers, Alan M.[3]; Tracy, William F.[1]
[1] University of Wisconsin-Madison; Moore Hall 1575 Linden Dr.; Madison, WI, 53705
[2] University of Florida; Fifield Hall 2550 Hull Road; Gainesville, FL, 32611
[3] Iowa State University; Molecular Biology 2437 Pammel Dr; Ames, IA, 50011

Starch comprises 60% of the maize endosperm weight and is composed of two glucan homopolymers, amylose and amylopectin. These polymers form insoluble granules and are synthesized by the coordinated activities of three families of starch biosynthetic enzymes: starch synthases, starch branching enzymes, and starch debranching enzymes (DBE). DBE are encoded by either one pullulanase, and/or three isoamylase type genes. The *sugary1 (su1)* gene encodes isoamylase1 (ISA1) and while a mutation at this locus can abolish ISA activity, pullulanase (PUL) activity is also decreased. The mechanism for this is not presently understood. Loss of *Su1* function leads to an increase in the soluble, highly branched glucan, phytoglycogen, that accumulates at the expense of amylopectin.

Insight into the mechanism by which loss of ISA1 reduces PUL activity has been gleaned from a long-term selection program. Seven cycles of divergent recurrent selection were performed on the base population Minn11 that is homozygous for the *su1-ref* allele. One direction of selection was for pseudostarchy, or plump kernels, characteristic of wildtype *Su1*, while the other direction of selection was for sugary kernels characteristic of *su1-ref*. Inbreds were developed from both populations following cycle 7. Two sugary inbreds (~200-300g mg^{-1} starch) and two pseudostarchy inbreds (~500-600g mg^{-1} starch) from this population, as well as parental controls, were assayed in 2015 and 2016 for enzymatic activities in the starch synthesis pathway on immature kernels at 21 days after pollination (DAP). While no differences were observed between the pseudostarchy and sugary inbreds for ADP-glucose pyrophosphorylase, starch synthase, or ISA activity, a significant increase in PUL activity was found in the pseudostarchy inbreds. The observed increase in PUL activity may be a key factor in the recovery of near-normal starch levels in the pseudostarchy lines, thus providing possible insights into semi-redundant functions within the starch biosynthetic pathway.

Funding acknowledgement: United States Department of Agriculture (USDA)

T10

tassels replace upper ears1 encodes a *BTB/POZ* ankyrin repeat gene that is directly targeted by *teosinte branched1*

(submitted by Zhaobin Dong <dongz@berkeley.edu>)
Full Author List: Dong, Zhaobin[1]; Li, Wei[2]; Vollbrecht, Erik[2]; Chuck, George[1]
[1] Plant Gene Expression Center, Plant and Microbial Biology Department, UC Berkeley, Albany, CA, USA, 94710
[2] Department of Genetics, Development and Cell Biology, Iowa State University, Ames, IA USA 50011

Axillary branch suppression is a common trait bred into many domesticated crop plants, including maize compared to its highly branched ancestor teosinte. Branch suppression in maize was achieved through selection of gain of function alleles of the *teosinte branched1* (*tb1*) transcription factor that acts as a repressor of axillary bud growth. Previous work indicated that other loci may function epistatically with *tb1*, and may be responsible for some of its phenotypic effects. Here we show that the *tassel replaces upper ears1* (*tru1*) mutant resembles the aerial portion of the *tb1* mutant, displaying long axillary branches tipped by tassels that replace ear primordia. The *tru1* gene was cloned by chromosome walking and found to encode an ankyrin repeat domain gene containing a *BTB/POZ* motif necessary for protein - protein interactions. Orthologues of *tru1* in monocots and dicots are necessary for the specification of basal leaf identities, indicating that *tru1* may play a role in tissue patterning and acquisition of cell fates. Immunolocalization using an antibody raised to TRU1 showed that the protein localizes to axillary buds in a pattern overlapping with *tb1*, consistent with it being a direct target. This was confirmed by epistasis, chromatin immunoprecipitation using a TB1 antibody, and gel shift analysis. A novel role for *tru1* in *tb1*-mediated domestication of maize was revealed by the identification of expression differences in the ear shanks of maize compared to teosinte. These results suggest that in domesticated maize the *tru1* mutant suppresses the effects of *tb1* overexpression, thus reverting maize back to its highly branched ancestral state.

Funding acknowledgement: National Science Foundation (NSF)

SESSION 4 – THE GENES THAT MAKE MAIZE II
Chair: Petra Wolters Friday, March 10. 4:40 PM – 6:00 PM

T11

Zmm22 gene in maize has pleiotropic effects on traits important for the production of food, feed, and fuel

(submitted by Mona Mazaheri <mmazaheri@wisc.edu>)
Full Author List: Mazaheri, Mona[1 2]; Burdo, Brett[1]; Heckwolf, Marlies[1 2]; Vaillancourt, Brieanne[3 4]; Gage, Joseph[1]; Buell, C. Robin[3 4]; de Leon, Natalia[1 2]; Kaeppler, Shawn M[1 2]
[1] Department of Agronomy, University of Wisconsin, Madison, Wisconsin 53706
[2] Department of Energy, Great Lakes Bioenergy Research Center, University of Wisconsin, Madison, Wisconsin 53706
[3] Department of Plant Biology, Michigan State University, East Lansing, Michigan 48824
[4] Department of Energy, Great Lakes Bioenergy Research Center, Michigan State University, East Lansing, Michigan 48824

Replacing fossil fuels with sustainable energy sources is a major current challenge. This challenge can be met by utilizing agricultural residues such as corn stover. With an annual production of about 300 million tons, corn stover is a major source of biofuel feedstock in the US. However, to replace 30% of transportation fuel with biofuels, stover biomass needs to be increased 40% by 2030. To achieve this goal, characterizing the genetic mechanism underlying stover biomass is essential. In this study, we investigated the natural genetic variation among maize inbred lines to detect candidate genes associated with biomass traits in genome-wide association studies (GWAS). In doing so, we assembled a panel of 835 inbred lines and phenotyped these lines for major biomass traits such as stalk diameter, plant height, leaf number, and flowering time. The population was genotyped with 430,947 RNA-Seq based single nucleotide polymorphism (SNP) markers. Our results showed that *zmm22* is the most significant candidate gene associated with all biomass traits measured in this study. To explain the pleiotropic effect of *zmm22*, we proposed that elevated expression of *zmm22* later in plant development triggers the suppression of vegetative growth and the activation of flowering transition. We supported this hypothesis by modifying the expression level of *zmm22*. Transgenic lines with high expression of *zmm22* had reduced vegetative growth; they flowered four days earlier and showed a decrease in stalk diameter, leaf number, and plant height by 14%, 10%, and 9% respectively, compared to the wild types. In turn, reduced expression of *zmm22* enhanced the biomass traits. Our results demonstrated that *zmm22* is a central gene in regulating maize development. Detecting this gene could open promising opportunities to increase stover biomass for sustainable energy production without compromising grain yield for food and feed.

Funding acknowledgement: Department of Energy (DOE)

T12

The maize *dek34-Dsg* encodes Tti2, a member of the TTT complex essential for PIKK stability

(submitted by Nelson Garcia <ngarcia@waksman.rutgers.edu>)
Full Author List: Garcia, Nelson[1]; Li, Yubin[1 2]; Dooner, Hugo[1]; Messing, Joachim[1]
[1] Waksman Institute at Rutgers University, Piscataway NJ, USA 08904
[2] (Present Address) Chinese Academy of Agricultural Sciences, Beijing, China

The TTT complex is a highly conserved co-chaperone in yeast and mammals. Composed of Tel2, Tti1, and Tti2, this complex is required to stabilize cellular levels of phosphatidylinositol 3-kinase-related kinase (PIKK) proteins such as TOR and ATM, which play essential roles in signaling pathways related to growth in response to nutrients and DNA damage response. Here, we report that the *dek34-Dsg* mutant in maize encodes a Tti2 homologue, the first member of the TTT complex to be characterized in plants. Similar to its yeast and mammalian homologues, maize Tti2 (ZmTti2) is required to maintain steady state levels of TOR and ATM proteins in maize. Embryo development is arrested early at the dermatogen stage, which mirrors the embryo arrest in the *Arabidopsis TOR* mutant, suggesting that embryo arrest in *dek34-Dsg* could be due to aberrant TOR function. Defective kernels from the *dek34-Dsg* mutant also accumulate less zein proteins, have smaller endosperm cells, and have a severely underdeveloped basal endosperm transfer layer (BETL) with little to no expression of BETL-specific genes. The reduction of ATM protein in the *dek34-Dsg* mutant also likely contributed to the pollen transmission defect observed because of ATM's important role in gametogenesis. We also cloned the *Tel2 (ZmTel2)* and *Tti1 (ZmTti1)* homologues in maize and showed via the yeast two-hybrid system that ZmTel2 can interact with both ZmTti1 and ZmTti2, indicating that their interaction and function are conserved from yeast to mammals and now in plants. Synteny analysis showed that the TTT complex and PIKK genes are single copy in maize, and were preferentially retained in the maize1 subgenome, indicating a tight co-evolution of interacting proteins that participate in the same pathway.

T13

Engineering amyloplast 6-phosphogluconate dehydrogenase activity to improve heat stability of the oxidative pentose phosphate pathway

(submitted by Camila Ribeiro <camila.ribeiro@ufl.edu>)
Full Author List: Ribeiro, Camila[2]; Myers, Alan M.[3]; Hennen-Bierwagen, Tracie[3]; Cline, Kenneth C.[1 2]; Tracy, William F.[4]; Boehlein, Susan D[1]; Hannah, L. Curtis[1 2]; Settles, A. Mark[1 2]
[1] Horticultural Sciences Department, University of Florida, Gainesville, Florida, 32611
[2] Plant Molecular and Cellular Biology Program, University of Florida, Gainesville, FL, 32611
[3] Department of Biochemistry, Biophysics and Molecular Biology, Iowa State University, Ames, Iowa, 50011
[4] Department of Agronomy, University of Wisconsin, Madison, Wisconsin, 53706

Heat stress reduces maize grain weight and quality. Starch synthesis in the endosperm is sensitive to high temperature stress and has the potential to be a limiting pathway for grain yield under heat stress. In addition to enzymes directly involved in starch biosynthesis, chloroplast-localized 6-phosphogluconate dehydrogenase (PGD3) is critical for starch accumulation. PGD3 is one of three enzymes in the oxidative section of the Pentose Phosphate Pathway (PPP). Maize encodes two cytosolic isozymes, PGD1 and PGD2. Double mutants of *pgd1; pgd2* have a nearly complete loss of cytosolic activity and develop normal kernels. We compared endosperm enzyme activity from the pgd3 mutant and *pgd1; pgd2* double mutants.Cytosolic PGD1 and PGD2 isozymes are heat stable, while the amyloplast-localized PGD3 is heat labile under in vitro and in vivo heat stress conditions. To develop a heat stable 6-phosphogluconate dehydrogenase localized to amyloplasts, we developed constructs to fuse the *waxy1* N-terminal chloroplast targeting sequence to the Pgd1 and Pgd2 open reading frames. The W::PGD1 and W::PGD2 fusion proteins import into isolated pea chloroplasts indicating that the targeting sequence is functional. Transgenic maize plants were generated to express W::PGD1 and W::PGD2 under the 27 kDa γ-zein promoter to confer endosperm specific expression. Transformants have increased 6-phosphogluconate dehydrogenase enzyme activity and isozyme activity assays suggest the increase is due to higher levels of PGD1 and PGD2. Transgenic endosperm shows enhanced heat stability in vitro. The W::PGD1 and W::PGD2 transgenes complement the pgd3 defective kernel phenotype suggesting the fusion proteins are targeted to the amyloplast. A preliminary field experiment suggests the W:PGD1 transgene can mitigate grain yield losses in heat stressed conditions. These data support a model in which the amyloplast PPP contributes to maize yield loss during heat stress.

Funding acknowledgement: United States Department of Agriculture (USDA), CNPQ-Brazilian National Council for Scientific and Technological Development

T14

Pectinmethylesterase (PME) is differentially expressed in *Ga1-S* silks and maps to a PME-repeat region near the *Ga1-S* locus

(submitted by Adrienne Moran Lauter <adrienne.moranlauter@ars.usda.gov>)
Full Author List: Moran Lauter, Adrienne N[1]; Muszynski, Michael G[2]; Scott, M Paul[1]
[1] USDA-ARS-CICGRU; Ames, Iowa 50011
[2] University of Hawaii at Manoa; Honolulu, Hawaii, 96822-2279

Maize gametophytic factors (*ga*) mediate the interactions between pollen and silk and determine the success of fertilization. The system consists of male and female components that together regulate pollen tube growth. Most dent and flint corn lines are *ga1/ga1* genotypes, and are cross-compatible. The *Ga1-S* allele is cross-incompatible with *ga1* genotypes. *Ga1-S* has been mapped to a 100 kb region of maize chromosome 4 based on B73 RefGen_v2 (Liu 2014). Despite only a few candidate genes in that region, the *Ga1-S* gene has yet to be identified. It is possible that *Ga1-S* is not present in *ga1* backgrounds, and therefore, identification through a candidate gene approach will not be effective. We began with a transcriptomic approach in order to understand the biological differences between *Ga1-S* and *ga1* silks. Of the top 20 most highly upregulated genes in *Ga1-S* silks, eleven mapped to chromosome 4 and all have significant homology to pectinesterase/pectinesterase inhibitor (PME/PMEI 38) genes. These genes all map within 2 Mb of the *Ga1-S* map position, but outside of the 100 kb region of interest. We used Trinity for *de novo* assembly of the RNAseq reads. In the *de novo* assembled transcripts, there is only one differentially expressed PME/PMEI-38 transcript and it was found exclusively in *Ga1-S* samples. Within a 2 Mb region of both W22 and B73 encompassing the *Ga1-S* map position, there are 59 unannotated full length or partial PME genes. All but two have pectinesterase/pectinesterase inhibitor 17/38 as their top BLAST hit. Using the ExPASy.org translate tool, we found that none of these genes have a full-length open reading frame while the Trinity-assembled transcript does.
Subsequent proteomic analysis has found that this predicted PME-38 encodes a protein detected in *Gas1-S* unpollinated and pollinated silks, but not in pollen or *ga1* unpollinated or pollinated silks.

Funding acknowledgement: United States Department of Agriculture (USDA)

T15

Maize imprinted gene *floury3* encodes a PLATZ protein required for tRNA and 5S rRNA transcription through interaction with RNA Polymerase III

(submitted by Qi Li <liqi01@sibs.ac.cn>)
Full Author List: Li, Qi[1]; Wang, Jiechen[1]; Ye, Jianwei[1]; Zheng, Xixi[1]; Xiang, Xiaoli[2]; Li, Changsheng[3]; Wang, Qiong[1]; Zhang, Zhiyong[1]; Wu, Yongrui[1]
[1] National Key Laboratory of Plant Molecular Genetics, CAS Center for Excellence in Molecular Plant Sciences, Institute of Plant Physiology & Ecology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai 200032, China
[2] Institute of Biotechnology and Nuclear Technology, Sichuan Academy of Agricultural Science, Chengdu 610061, China
[3] College of Agronomy, Shenyang Agricultural University, Shenyang 110866, China

Maize *floury3 (fl3)* is a classic semi-dominant negative mutant that shows severe defects in endosperm but appears normal in embryo and plant. The mutant phenotype only occurs when *fl3* is transmitted through the female. We cloned the gene and found that it encodes a PLATZ (PLant-specific AT-rich and Zinc-bind) protein. *Fl3* is specifically expressed in the starchy endosperm. It revealed that *fl3* results from a point mutation, leading to the Arg to His replacement in the PLATZ domain. Protein sequence alignment of Fl3 alleles from 165 inbred lines and its orthologues from other species demonstrated that the Arg residue is extremely conserved, indicating that this amino acid is critical for Fl3's function. Transformation of *fl3* in maize and rice reproduced the same floury phenotype in seeds, confirming the cloned gene being *Fl3*. We also investigated the expression levels of the *Fl3* and *fl3* alleles and their methylation status in their reciprocal crosses and found this gene is subject to imprinting, leading to a suppressed expression of *fl3* when transmitted from the male, which may partially explain its semi-dominant behavior in the genetic analysis. Yeast two-hybrid screening and BiFC revealed that FL3 protein is able to interact with RPC53 and TFC1, two critical components of RNA polymerase III transcription complex. In *fl3*, the levels of many of tRNAs and 5S rRNA transcribed by RNA polymerase III are significantly reduced, suggesting that the incorrect fl3 protein may affect their biogenesis. The transcriptome was dramatically altered in *fl3*, in which the differentially expressed genes are mainly enriched to pathways related to the translation, ribosome, misfolded protein responses and nutrient reservoir activity. As a consequence, these changes may lead to defects in the endosperm development and storage reserve filling in *fl3*.

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T16

An expression atlas of the maize shoot apex reveals signatures of TFs as important drivers for cell identity and sub-functionalization of key downstream factors

(submitted by Steffen Knauer <sknauer@cshl.edu>)
Full Author List: Knauer, Steffen[1]; Javelle, Marie[1]; Li, Lin[2]; Li, Xianran[3]; Wimalanathan, Kokulapalan[4]; Kumari, Sunita[1]; Leiboff, Samuel[5]; Johnston, Robyn[5]; Ware, Doreen[1]; Lawrence, Carolyn J[4]; Schnable, Patrick S[6]; Yu, Jianming[3]; Muehlbauer, Gary J[2]; Scanlon, Michael J[5]; Timmermans, Marja C[1]
[1] Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 11724, USA
[2] Department of Agronomy and Plant Genetics, University of Minnesota, Saint Paul, MN 55108, USA
[3] Department Agronomy, Iowa State University, Ames, IA 50011, USA
[4] Department of Genetics, Development and Cell Biology and Department of Agronomy, Iowa State University, Ames, IA 50011, USA
[5] Department of Plant Biology, Cornell University, Ithaca, NY 14853, USA
[6] Center for Plant Genomics, Iowa State University, Ames, IA 50011, USA

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 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 merely ~10% of all transcribed genes are differentially expressed across these tissue types, and identified a valuable collection of cell type specific genes. Interestingly, very few functional categories known to function in stem cell maintenance and organogenesis are enriched among the differentially expressed genes, which we show, reflects prominent sub-functionalization within gene families. However, strong enrichments were seen for transcription factor (TF) families, and principle component analysis on all expressed TFs identified unique TF signatures predictive of meristematic and vascular fate. Natural variation at TFs correlated with these two tissues is associated with key plant architectural traits in GWAS, providing functional support for our findings. Moreover, analysis 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 pattern of expression. Additionally, we show that the TF KNOTTED1, a key meristem determinant, acts mainly by inhibiting organogenesis and differentiation at the tip of the SAM. Our data set also allowed us to define functional domains within the SAM, namely the stem cell harboring central zone, the peripheral zone flanking the stem cells and an organizing center located directly underneath the stem cell population. Genes in these zones are in part conserved among maize and Arabidopsis, but our data also emphasize remarkable differences and novel gene functions in maize. Phenotypic characterizations of mutants confirm a role for SAM tip specific genes in regulation of SAM architecture. In summary, our findings present a novel approach to modulating plant architecture by manipulating master regulators of cell identity.

Funding acknowledgement: National Science Foundation (NSF), German Research Foundation

T17

Contribution of translational control to establishing the distinct proteomes of bundle sheath and mesophyll cells in maize

(submitted by Prakitchai Chotewutmontri <pchotewu@uoregon.edu>)
Full Author List: Chotewutmontri, Prakitchai[1]; Stiffler, Nicholas[1]; Kumar, Indrajit[2]; Brutnell, Thomas[2]; Barkan, Alice[1]
[1] Institute of Molecular Biology, University of Oregon, Eugene, OR 97403 USA
[2] Donald Danforth Plant Science Center, St Louis, MO 63132 USA

C4 photosynthesis in maize occurs by partitioning photosynthesis between two morphologically and functionally-distinct cell types, bundle sheath (BS) and mesophyll (M) cells. The distinct proteomes of BS and M cells (Majeran et al, Plant Cell 2010) result in part from distinct transcriptomes (Li et al Nat Genet 2010; Tausta et al, J Exp Bot 2014). However, little attention has been paid to the possibility that translational regulation contributes as well. We are using ribosome profiling to provide a genome-wide view of the contribution of differential translation to establishing the distinct proteomes of BS and M cells. Ribosome profiling uses deep-sequencing to map ribosome footprints on mRNAs. Normalization of ribosome footprint abundance to RNA-seq data is used to infer translational efficiencies.

We established a rapid mechanical fractionation procedure that results in highly enriched BS and M fractions within minutes of tissue harvest. RNA-seq data from these fractions correlates well with those reported for BS and M fractions recovered by laser capture microdissection (Tausta et al, 2014). We found that differential expression of chloroplast genes in BS and M cells results primarily from differences in mRNA abundance, but differences in translational efficiency amplify mRNA-level effects in some instances (Chotewutmontri, Barkan, PLOS Genet 2016). Analysis of the cytosolic data is in progress, but results so far suggest that differences in translational efficiency contribute to the differential expression of roughly half of the genes whose translational output differs by > 2-fold in BS and M cells. In some cases, differences in translational efficiency amplify the effects of differential RNA accumulation, whereas in others, differences in translational efficiency dominate. Differences in mRNA abundance are buffered by the opposite change in translational efficiency for a small set of genes. We are currently seeking biological correlations with these distinct behaviors and clues as to the underlying mechanisms.

Funding acknowledgement: National Science Foundation (NSF)

T18

Highly-interwoven communities of gene regulatory network unveil crucial genes for maize seed development

(submitted by Wenwei Xiong <xiongwe@mail.montclair.edu>)
Full Author List: Xiong, Wenwei[1]; Wang, Chunlei[2]; Zhang, Xiangbo[2]; Yang, Qinghua[3]; Shao, Ruixin[3]; Lai, Jinsheng[2]; Du, Chunguang[1]
[1] Department of Biology, Montclair State University, Montclair, NJ 07043
[2] National Maize Improvement Center, China Agricultural University, Beijing 100083, China
[3] National Key Laboratory of Wheat and Maize Crop Science, Henan Agricultural University, Zhengzhou 450002, China

The complex interactions between transcription factors (TFs) and their target genes essentially govern the dynamic process of maize seed development. Topological properties of genes in the regulatory network often reflect the importance of their roles and contributions. Identifying the most important players in maize seed development can have profound implications in our understanding of the genetic control as well as agriculture. Here we aim to achieve this goal through a data mining approach that integrates spatial-temporal RNA-Seq profiles, microdissected compartments data of maize seed, and manually curate KEGG pathways. First, we reverse engineered a regulatory network from genes as nodes and their interactions as edges based on information theory. Then we studied collective gene interaction patterns and uncovered highly-interwoven network communities as the building blocks of the regulatory network. We propose that communities with the highest connectivity and scores are crucial to sustain the network integrity. Here we discovered two such high-profile communities. One consists of mostly unknown genes interacting with well-studied *bt1, o2* and *shrunken2*. Their tight interactions may contribute to various kernel phenotypes. Second community has a surprising 83% of genes located in the basal endosperm transfer layer. Furthermore, we found that top highly-connected network hubs tend to collaborate. We also provides 73 new candidate genes as direct targets of *o2*, among many other known/unknown TFs. We identified non-TF genes with high collective-influence, and found that they are mostly regulated by hub TFs, e.g. two top unknown genes from the conducting zone share regulators with the *zein* genes. To ensure our network reliability, we further verified a subset of predicted TF-gene bindings with our yeast one hybrid assays as well as published ChIP-Seq data. In conclusion, this study uncovers genes and functional units as communities working collaboratively that are crucial to maize seed development.

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T19

Functional analysis of genetic variation of the brassinosteroid *cis*-regulatory network using hybrid allele-specific ChIP-Seq

(submitted by Thomas Hartwig <thartwig@carnegiescience.edu>)
Full Author List: Hartwig, Thomas[1]; Banf, Michael[1]; Rhee, Seung Y.[1]; Wang, Zhiyong[1]
[1] Carnegie Institution for Science, 260 Panama Street, Stanford, 94305, USA

Cis-regulatory elements such as transcription factor (TF) binding sites can be identified genome-wide, but it remains far more challenging to pinpoint genetic variants affecting TF binding. We developed Hybrid Allele-Specific ChIP-seq (HASCh-Seq) to identify natural variations that affect TF affinity *in vivo*, and used it to functionally analyze the brassinosteroid (BR) *cis*-regulated network in maize. Specifically, we performed ChIP-seq of the BR-responsive TF BZR1 in B73 and reciprocal F1 hybrids of B73 and Mo17. These experiments identified 6000 putative BZR1 target genes, of which 1200 were BR-responsive in RNA-seq. In addition, HASCh-Seq mapped 4500 independent allele-specific binding (ASB) loci that quantitatively affect BZR1 affinity between B73 and Mo17. Majority of ASB events (70%) localized to *cis*-regulatory regions, were overrepresented (22%) in the core BZR1 *cis*-element CGTG, and their effect on affinity predictable in 86% of those sites. Hundreds of ASBs have been implicated in genome-wide association studies, providing candidate molecular mechanisms for complex traits with known phenotypic variance between B73 and Mo17, such as tassel branching and northern leaf blight resistance. Interestingly, about 200 ASBs showed parent-of-origin–dependent BZR1 binding, which indicates that epigenetic variation influences TF affinity. Together our results suggest that TF binding variation may underlie a large fraction of maize phenotypic variation.

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T20

Evolutionarily conserved role of a novel RNA binding protein involved in the splicing of U12-type introns

(submitted by Amy Siebert <aesieber@oakland.edu>)
Full Author List: Siebert, Amy E.[1]; Gronevelt, J. Paige[1]; Kenney, Catalina V.[1]; Davenport, Ruth[2]; Barbazuk, W. Brad[2]; Westrick, Randal J.[1]; Settles, A. Mark[2]; Lal, Shailesh K.[1]; Madlambayan, Gerard J.[1]
[1] Department of Biological Sciences, Oakland University, Rochester, MI, 48309
[2] Department of Horticultural Sciences, University of Florida, Gainesville, FL, 32611

U12-type introns constitute a distinct group of introns that have been identified in the majority of eukaryotic genomes, including those of plants and animals, and are spliced by a minor spliceosome. Constituting only up to 0.5% of all introns, they have been shown to have roles in growth and development. Recently, a novel maize RNA binding motif protein 48 (RBM48) was identified and found to be involved in U12 intron splicing. Mutants of maize *rbm48*, with abnormal endosperm cell differentiation and proliferation, show genome-wide aberration of U12 intron splicing. Maize *rbm48* is orthologous to human *RBM48* and aberrant U12 intron retention has been shown to affect similar cellular processes in both normal human hematopoietic stem/progenitor and myelodysplastic cells. We investigated whether human RBM48 has conserved functional effects on U12 intron splicing and concomitant cellular function. Using RT-PCR, we found that all tested human cell types (normal bone marrow and cell lines derived from hematological malignancies) express *RBM48* transcripts. To investigate whether the role of RBM48 in U12 intron splicing is conserved between maize and humans, we generated a CRISPR/Cas9-mediated *RBM48* functional knockout ($RBM48^{FunKO}$) in human K-562 cells. To define genes impacted by $RBM48^{FunKO}$, we identified U12-containing human genes that have orthologs in maize and are involved in cell cycle regulation, DNA repair and chromatin dynamics. RT-PCR analysis of $RBM48^{FunKO}$ cells revealed aberrant splicing or differential expression of U12-type introns in all six genes selected for comparison to vector controls. Ongoing RNAseq analysis will confirm U12 intron retention, identify additional U12 intron-containing genes, and determine if these genes show homology with maize. Overall, these results indicate evolutionarily conserved functions for maize *rbm48* and human *RBM48*. Identifying the specific role of RBM48 will provide insights into the role of U12 introns and minor spliceosome activity in normal and malignant cell function.

Funding acknowledgement: National Institutes of Health (NIH), National Science Foundation (NSF), Research Excellence Fund (REF)

T21

Regulation of vacuolar trafficking by autophagy in maize aleurone cells

(submitted by Marisa Otegui <otegui@wisc.edu>)
Full Author List: Zhang, Xiaoguo[1]; Ding, Xinxin[1]; Marshall, Richard[2]; Vierstra, Richard[2]; Otegui, Marisa[1]
[1] Department of Botany and Laboratory of Cell and Molecular Biology; University of Wisconsin, Madison, Wisconsin 53706, USA
[2] Department of Biology, Washington University in St. Louis, St. Louis, Missouri 63130, USA

The aleurone in maize is a single layer of cells that represent the endosperm epidermis. Aleurone cells accumulate storage compounds such as proteins, lipids, and over 70% of the mineral stores (phosphate, magnesium, potassium, iron, and calcium) of the whole endosperm. A central organelle in aleurone cells are the protein storage vacuoles (PSVs) that contain storage proteins and other compounds with important nutritional and health-promoting properties. We have performed structural analysis by electron tomography and live cell imaging to investigate the underlying trafficking mechanisms that control PSV assembly in aleurone cells. To further understand these pathways, we identified genes highly expressed in aleurone cells between 15 and 22 days after pollination (DAP) and analyzed relevant Mu-insertional mutants for aleurone cellular defects. One of these candidate genes encodes a protein predicted to localize to the endoplasmic reticulum (ER) and to remodel the shape of ER membranes. We found that two independent mutations in this gene (*rtn2-1* and *rtn2-3*) drastically affect the delivery of ER and other organelles to PSV by a mechanism called autophagy. Autophagy controls the delivery of cytoplasmic components (including organelles) to the vacuole, where they are degraded. The introduction of a mutation in *Atg12*, a gene required for autophagy, in the *rtn2* mutants completely suppressed the abnormal content accumulation in aleurone PSVs. Autophagy receptors are proteins that select autophagy cargo and interact with ATG8, a key component for the progression of autophagy. We found that the identified ER-localized protein interacts with a maize ATG8 protein, suggesting that it could have a direct role in regulating the autophagic turnover of the ER. A model to explain the intersection of multiple vacuolar trafficking pathways in aleurone cells will be discussed.

Funding acknowledgement: National Science Foundation (NSF), United States Department of Agriculture (USDA), Hatch project WIS01791

T22

The mitosis-to-meiosis transition of pollen mother cells is controlled independently by *Mac1* and *Am1* in maize

(submitted by Rachel Wang <rwang@gate.sinica.edu.tw>)
Full Author List: Tseng, Ching-Chih[1]; Chen, Shu-Yun[1]; Ku, Jia-Chi[1]; Wang, Chi-Ting[1]; Kao, Yu-Hsin[1]; Wang, CJ Rachel[1]
[1] Institute of Plant and Microbial Biology, Academia Sinica, Taipei, 11529, Taiwan

The transition from mitosis to meiosis is a defining juncture in the life cycle of sexually reproducing organisms. How the mitosis is switched to meiosis in plants remains unknown. Here, by labeling DNA replication, we unveil that in maize anthers, differentiated male germ cells first undergo asynchronous mitosis with a gradually decreased mitotic rate until a cell cycle resting stage in all PMC cells. Next, the pre-meiotic S is initiated synchronously, followed by the prophase I. In contrast, we show that multiple archesporial cells1 (mac1) mutant lacks the resting stage and continuous mitotic cell divisions result in extra PMCs. Nearly 35% of mac1 meiocytes successfully enter meiosis and reach prophase I; however, their meiosis is less synchronous. The rest 65% of mac1 meiocytes undergo mitosis or arrest at interphase. The ameiotic1 (am1) mutant, on the other hand, fails to enter the pre-meiotic S phase. After a prolonged resting stage, meiocytes resume asynchronous mitosis. We propose that the secreted protein MAC1, which is important for the balance between germ cells and soma development, promotes the resting stage of male germ cells. Within a short period of the pause, AM1 enables these resting meiocytes to enter the pre-meiotic S phase synchronously. Double mutant of mac1 am1 exhibited additive phenotypes, suggesting two independent pathways are required to establish meiotic synchrony and meiotic initiation. Finally, transcriptomic and proteomic analyses of isolated meiocytes identified other candidate genes that may be involved in this transition.

Funding acknowledgement: Academia Sinica

T23

A combination of pharmacological approaches and genetics provides insights into benzoxazinoid exudation by young maize roots

(submitted by Claudiu Niculaes <claudiu.niculaes@tum.de>)
Full Author List: Niculaes, Claudiu[1]; Robert, Christelle[2]; Bauer, Eva[1]; Erb, Matthias[2]; Frey, Monika[1]
[1] Technical University of Munich, Chair of Plant Breeding; Liesel-Beckmann-Str. 2; Freising; Germany; D-85354
[2] University of Bern, Institute of Plant Sciences Biotic Interactions; Altenbergrain 211; Bern; Switzerland; CH-3013

Benzoxazinoids are defense compounds produced in large amounts by young maize plants. These compounds are also present in maize root exudates, but the mechanism by which benzoxazinoid exudation takes place has remained obscure. In order to address this question, we first developed a method that allows exudate collection from young maize roots without damage to the root tissue. Then, by combining pharmacological approaches with QTL mapping, we were able to bring the first insights into benzoxazinoid exudation. Our findings suggest that maize roots accumulate and exude benzoxazinoids in a different manner than what has been described for other defense compounds. At the same time, we observed that the exudate composition is linked to genetically controlled differences in the benzoxazinoid composition of the root tissues. These differences allowed QTL fine-mapping to a region small enough to reveal candidate genes. Interestingly, the QTL region does not include the main benzoxazinoid biosynthesis gene cluster. Therefore, this approach has the potential to identify new players that could be involved in benzoxazinoid biosynthesis, stability, regulation or transport.

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T24

Comparative phosphoproteomic analysis of immunoregulatory signaling in maize and arabidopsis

(submitted by Alisa Huffaker <ahuffaker@ucsd.edu>)
Full Author List: Huffaker, Alisa[1]; Weckwerth, Philipp[1]; Dressano, Keini[1]; Shen, Zhouxin[1]; Briggs, Steve[1]
[1] Section of Cell and Developmental Biology, University of California, San Diego, La Jolla, CA, 92014

Plants recognize attacking organisms and counter with broadly protective innate immune responses to defend themselves. This occurs through activation of signaling networks that trigger downstream defense responses, with initial signaling events often mediated through rapid post-translational modifications of protein components. To identify post-translationally regulated immunomodulatory proteins and their target modification sites, we profiled changes in the phosphoproteome within minutes of treatment with peptide signals that elicit defense responses. Plant elicitor peptides (Peps) have demonstrated roles as endogenous inducers of innate immunity in both Maize and Arabidopsis. We treated both species with their cognate Peps and analyzed subsequent changes in protein phosphorylation. This phosphoproteomic screen revealed some proteins already implicated in plant innate immunity, but yielded predominantly proteins that have not yet been associated with this response. Many classes of protein were found to change in phosphorylation state, from transcription factors and ion channels to secretory proteins and cytoskeletal components. We're focused on characterization of RNA and DNA binding proteins, and have uncovered several new candidate positive and negative regulators of immune responses in both species. The mechanisms by which these proteins function is currently under study both biochemically and genetically. Together these analyses allow comparison of monocot and dicot signaling cascades that contribute to defense responses and provide novel targets for manipulation of resistance to both pests and pathogens.

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T25

Crosstalk between 9-oxylipins and jasmonates pathways modulates the maize resistance to stem rot caused by *Fusarium graminearum*

(submitted by Xiquan Gao <xgao@njau.edu.cn>)
Full Author List: Wang, Shi[1]; Yang, Yang[1]; Kolomiets, Michael V.[2]; Gao, Xiquan[1]
[1] State Key Laboratory of Crop Genetics and Germplasm Enhancement, College of Agriculture, Nanjing Agricultural University, Nanjing, Jiangsu Province 210095, China.
[2] Department of Plant Pathology and Microbiology, Texas A&M University, 2132 TAMU, College Station, 77843-2132, USA.

As one of the major types of oxylipins (oxygenated poly-unsaturated fatty acid) formed by plant 13-lipoxygenase pathways, jasmonates (JAs) have been well-known for their functions in regulating plant development and stress-related processes. The function of 9-LOXs and 9-oxylipins, however, remains largely unexplored. We previously reported that 9-oxylipin pathways could mimic or interfere with fungal oxylipins to modulate the interaction between maize and fungal pathogens, and JA pathways played essential role in the immunity to *Fusarium verticilliodes*. Similar to *F. verticilliodes*, *Fusarium graminearum*, also significantly impacts maize production worldwidely, particularly in China, by causing ear rot and stalk rot. To address how maize 9-oxylipins coordinate with JA pathways to regulate the resistance to *F. graminearum*, we deployed the combination of genetic, genomic and biochemical approaches to investigate the role of 9-LOX genes, JAs and other potential immune genes in the responses to this pathogen. While we found a dynamics and signaling complex mediated by 9-LOXs and JAs in the resistance to stalk rot caused by *F. graminearum*, other potential immune signaling components are also likely involved in such immunity mediated by oxylipins. The possible mechanism underlying the oxylipins-mediated resistance to *F. graminearum* will be discussed.

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T26

A *caffeoyl-CoA O-methyltransferase* gene confers quantitative resistance to multiple pathogens in maize

(submitted by Qin Yang <qyang6@ncsu.edu>)
Full Author List: Yang, Qin[1]; He, Yijian[1]; Kabahuma, Mercy[2]; Kelly, Amy[1]; Chaya, Timothy[3]; Borrego, Eli[4]; Bian, Yang[5]; Kasmi, Farid El[6]; Yang, Li[6]; Kolkman, Judith[7]; Nelson, Rebecca[7]; Kolomiets, Mike[4]; Dangl, Jeffery[6]; Wisser, Randall[8]; Caplan, Jeffrey[3]; Li, Xu[9]; Lauter, Nick[2 10]; Balint-Kurti, Peter[1 11]
[1] Department of Entomology and Plant Pathology, North Carolina State University, Raleigh, NC, USA 27695
[2] Department of Plant Pathology and Microbiology, Iowa State University, Ames, IA, USA 50011
[3] Department of Biological Sciences, University of Delaware, Newark, DE, USA 19716
[4] Department of Plant Pathology and Microbiology, Texas A&M University, College Station, TX, USA 77843
[5] Department of Crop Science, North Carolina State University, Raleigh, NC, USA 27695
[6] Department of Biology, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA 27599
[7] School of Integrative Plant Science, Cornell University, Ithaca, NY, USA 14853
[8] Department of Plant and Soil Sciences, University of Delaware, Newark, DE, USA 19716
[9] Plants for Human Health Institute, North Carolina State University, Kannapolis, NC, USA 28081
[10] USDA-ARS Corn Insects and Crop Genetics Research Unit, Ames, IA, USA 50011
[11] USDA-ARS Plant Sciences Research Unit, Raleigh, NC, USA 27695

Alleles that confer multiple diseases resistance are valuable in crop improvement though molecular mechanisms underlying functions remain largely unknown. Southern leaf blight (SLB, causal agent Cochliobolus heterostrophus), gray leaf spot (GLS, causal agent Cercospora zeae-maydis) and northern leaf blight (NLB, causal agent Exserohilum turcicum) are three major economically damaging foliar fungal diseases in maize worldwide. A QTL, qMdr9.02, associated with resistance to SLB, GLS and NLB had been identified on maize chromosome 9. Through fine mapping, association analysis, expression analysis, insertional mutagenesis, and transgenic validation, we demonstrate that ZmCCoAOMT2, which encodes a caffeoyl-CoA O-methyltransferase associated with the phenylpropanoid pathway and lignin production, is the gene within qMdr9.02 conferring quantitative resistance to both SLB and GLS but not to NLB. Near isogenic lines (NILs) differing for the resistance/susceptibility qMdr9.02 allele were constructed. Transcript levels of ZmCCoAOMT2 were significantly induced in the resistant but not the susceptible NIL and the lines displayed differential levels of lignin and metabolites associated with the phenylpropanoid pathway. We had previously shown that ZmCCoAOMT2 alters the hypersensitive defense response (HR) induced by an auto-active resistance protein Rp1-D21. Here we show ZmCCoAOMT2 to be a general suppressor of HR with the resistance allele having a stronger suppressive effect than the susceptibility allele. These results suggested that resistance might be caused by allelic variation at the levels of both gene expression and amino acid sequence causing differences in levels of lignin and other metabolites of the phenylpropanoid pathway and regulation of the HR respectively. The moderate effect of qMdr9.02, exert low selection pressure, combined with its broad-based modes of action, would be expected to make this QTL quite durable.

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T27

Corn smut sugar pathophysiology detected by live FRET imaging

(submitted by Davide Sosso <dsosso@carnegiescience.edu>)
Full Author List: Sosso, Davide[1]; Van Der Linde, Karina[2]; Greenfield, Margaret[1]; Schuler, David[3]; Kämper, Jörg[3]; Frommer, Wolf B.[1 2]; Walbot, Virginia[2]
[1] Department of Plant Biology, Carnegie Science, Stanford, CA 94305, USA
[2] Department of Biology, Stanford University, Stanford, CA 94305, USA
[3] Department of Genetics, Institute of Applied Biosciences, Karlsruhe Institute of Technology, Karlsruhe 76187, Germany

The basidiomycete *Ustilago maydis* is the causal agent of corn smut disease in maize (*Zea mays L. ssp. mays*) where it infects all aerial vegetative and floral organs, generating large chlorotic areas and extensive tumors. Typical of pathogens, *Ustilago* must access plant nutrients, primarily sugars, stored within host cells. The dynamics of plant and *Ustilago* rivalry for sugars have not yet been elucidated. Here, we demonstrate that *Ustilago* negatively impacts maize yield, diverting sugars from the developing ears to infected tassels and leaves, generating sugar-rich niches for fungal development. The mechanism of sugar diversion is likely achieved by hijacking maize sugar transporters, specifically downregulating *ZmSUT1* to block leaf sugar translocation, and upregulating three *SWEET* sugar transporter genes to increase soluble sugar concentrations in the apoplasm. We deployed a cytosolic glucose FRET sensor in *Ustilago* to monitor fungal uptake and metabolism, and found a polarized glucose distribution within the hypha with possible implications for both fungal sugar transport and metabolism. FRET sensor sugar monitoring provides new insights into maize:*Ustilago* interaction, and the approach should be applicable to other plant or animal host:pathogen systems.

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T28

Genetic control of maize biochemical defense against *Fusarium graminearum* seedling blight.

(submitted by Shaoqun Zhou <sz357@cornell.edu>)
Full Author List: Zhou, Shaoqun[1 2]; Jander, Georg[2]
[1] School of Integrated Plant Sciences, Cornell University, 412 Mann Library Building, Ithaca, NY. USA 14850
[2] Boyce Thompson Institute, 533 Tower Rd., Ithaca, NY USA 14850

Fusarium graminearum is a widespread fungal pathogen attacking grain crops. In maize (*Zea mays*), it is a major concern due to mycotoxin contamination of kernels, and negative effects on yield from stem rot and seedling blight. Previous genetic mapping of *F. graminearum* resistance in maize identified a large number of environment-dependent quantitative trait loci (QTL) with small effect sizes. In this study, we found that *F. graminearum*-resistant maize inbred line Mo17 constitutively accumulates many metabolites that are inducible by the same pathogen strain in the susceptible inbred line B73. We hypothesized that the contrasting *F. graminearum* susceptibility levels of these two inbred lines could be attributed to differences in their constitutive biochemical defense, and further, to the genes regulating the accumulation of defense-related metabolites. Therefore, we conducted non-targeted metabolite profiling by UPLC-MS and RNAseq transcript profiling of 80 B73 x Mo17 recombinant inbred lines, thereby identifying significant metabolite QTL (mQTL) for over 700 mass features that show constitutive differences between B73 and Mo17 seedling roots. Through this analysis, we identified an mQTL hotspot affecting over 70 mass features, including ones known to be defense-related. To pinpoint the causative gene(s) underlying this mQTL hotspot, we performed a correlative network analysis across the metabolomic and transcriptomic data, thereby identifying a gene that likely encodes a vesicular transport protein as the most probable candidate. Transposon insertions in this gene result in significant depletion of DIMBOA-glucoside, a major defense-related metabolite in maize seedling roots, which could compromise defense against F. graminearum. In summary, we show that maize biochemical defense against a fungal pathogen may be regulated by a vesicular transport protein, highlighting the importance of cellular trafficking in plant secondary metabolism. We also demonstrate the power of combining high-throughput transcriptomics, metabolomics, and quantitative genetics to achieve a system-level understanding of plant metabolism and biotic interactions.

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T29

A forward genetics approach to explore natural variation for enhancer/suppressors identifies components of the guard strategy of plant immunity

(submitted by Ross Zhan <rzhan@purdue.edu>)
Full Author List: Zhan, Ross[1]; Leonard, April[2]; Singh, Akanksha[1]; Best, Norman[3]; Benke, Ryan[4]; Carraro, Nicola[5]; Li, Bailin[2]; Multani, Dilbag[2]; Dilkes, Brian[4]; Johal, Guri[1]
[1] Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN, 47906
[2] Dupont Pioneer, Johnston, IA, 50131
[3] Department of Horticulture, Purdue University, West Lafayette, IN, 47906
[4] Department of Biochemistry, Purdue University, West Lafayette, IN, 47906
[5] Department of Agronomy, Purdue University, West Lafayette, IN, 47906

The focus of this talk is a lesion mimic mutant, *les23* and its suppressor, *Slm1* (suppressor of lesion mimics-1). We previously cloned *Slm1* and *les23* and found *Slm1* to be a defective R gene and *les23* a homolog of Arabidopsis *Rin4*. Originally identified as an interactor of the R protein RPM1, RIN4 has emerged as a key component of the guard mechanism of plant innate immune responses. Pathogen-induced phosphorylation or degradation of RIN4 activates the R proteins RPM1 and RPS2, respectively, leading to a robust hypersensitive cell death (HR) response. In this regard, it is possible that the mutation in *les23* causes a conformation change that results in loss of interaction with *Slm1* and induces HR. However, if *Slm1* is non-functional, as it is in the *les23*-suppressing QTL *Slm1*, no cell death is initiated whether *les23* is defective or not. Interestingly, in the NAM founders, 7 lines have a truncated *Slm1*, 6 lines have a *Slm1* identical sequence to B73, and 12 lines have a *Slm1* that contains multiple SNPs and small indels. Using the heterologous system *N. benthamiana*, we were able to reconstitute the *les23/Slm1* system and found that expressing SLM1 by itself is sufficient to cause HR, similar to RPS2. The HR induced by SLM1 is suppressed when WT LES23 is co-expressed but not by mutant LES23. Recently, we identified a homeolog of *les23* on chromosome 10 which we have designated *ltl1* (*les23*-like 1). *ltl1* is highly similar in sequence to *les23*, differing by only 28 amino acids and when mutated, makes the *les23* phenotype more severe. LTL1-Va35 is capable of suppressing HR induced by SLM1 in *N. benthamiana*. Three other paralogs of *les23* are also present in maize and their function is being examined.

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T30

Testing hypotheses on adaptation with exotic allele series at photoperiod loci in maize

(submitted by David Wills <David.Wills@ars.usda.gov>)
Full Author List: Wills, David M.[1 2]; Lauter, Nick[3 4]; Weldekidan, Teclemariam[5]; Lopez, Miriam[3 4]; de Leon, Natalia[6]; Flint-Garcia, Sherry[1 2]; Holland, James B.[7 8]; Murray, Seth C.[9]; Xu, Wenwei[10]; Wisser, Randall J.[5]
[1] USDA-ARS, Plant Genetics Research Unit, Columbia, MO
[2] Division of Plant Sciences, University of Missouri, Columbia, MO
[3] USDA-ARS, Corn Insects and Crop Genetics Research Unit, Ames, IA
[4] Department of Plant Pathology and Microbiology, Iowa State University, Ames, IA
[5] Department of Plant and Soil Sciences, University of Delaware, Newark, DE
[6] Department of Agronomy, University of Wisconsin, Madison, WI
[7] USDA-ARS, Plant Science Research Unit, Raleigh, NC
[8] Department of Crop Science, North Carolina State University, Raleigh, NC
[9] Department of Soil and Crop Sciences, Texas A&M University, College Station, TX
[10] Lubbock Research and Extension Center, Texas A&M AgriLife Research, Lubbock, TX

Flowering time can act as a strong barrier to the adaptation of a crop to a new environment. For tropical maize, flowering time is typically delayed in long day environments. This photoperiod response may mask the effects of beneficial alleles via pleiotropy, and the selection required for flowering time adaptation may result in a loss of beneficial alleles in coupling phase with photoperiod sensitive haplotypes. To address questions about the nature of pleiotropy, linkage drag, and allelic variation in terms of environmental adaptation, we created a population design constructed as a set of near-isogenic lines capturing an allelic series (NILAS). A NILAS is a collection of inbred lines differing at a single specific genomic locus for a range of functional alleles. Marker-assisted selection was used to construct NILAS for each of four photoperiod quantitative trait loci (QTL) implicated as primary barriers to maize adaptation to temperate environments. Fourteen distinct tropical x temperate NILAS contrasts were created using seven tropical inbred lines as allele donors and two different temperate inbred lines as genetic backgrounds. Each NILAS captured 12 overlapping introgressions tiled across the QTL region allowing hypothesis testing of pleiotropy versus linkage drag during adaptation. The NILAS were assessed in eight environments over two years extending from approximately 18° N latitude (Puerto Rico) to approximately 43° N latitude (Wisconsin) in two replicates of a four-way split plot design. We present evidence for an allele series, phenological pleiotropy, and linked variation based on the evaluation of >20 characteristics. The NILAS is a powerful design to phenotypically, genetically, and ecologically characterize genomic loci. Our findings demonstrate the complexity of quantitative variation within a chromosome based on multi-environment trials and how the NILAS design is extensible for continued studies.

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T31

Revealing the regulatory impacts of rare variants in maize with low cost RNASeq

(submitted by Karl Kremling <kak268@cornell.edu>)
Full Author List: Kremling, Karl A[1]; Chen, Shu Yun[2]; Su, Mei Hsiu[2]; Swarts, Kelly[1]; Lepak, Nicholas[3]; Romay, M. Cinta[2]; Lu, Fei[2]; Bradbury, Peter[1 3]; Buckler, Edward[1 2 3]
[1] Department of Plant Breeding and Genetics, Cornell University, Ithaca, NY 14853
[2] Institute of Biotechnology, Cornell University, Ithaca, NY 14853
[3] USDA-ARS, Cornell University, Ithaca, NY 14853

Replicating the 2.5 billion base-pair maize genome for thousands of generations results in the accumulation of new mutations. Mutations derived from this process can be deleterious and impact fitness by contributing to disease and inbreeding depression. Although selection acts to keep these variants rare, their complete removal is impaired by physical linkage to favorable loci and finite population size, which contributes to genetic drift. For over one hundred years, maize breeders have systematically reduced the effects of this constant mutational pressure through strong artificial selection and self-fertilization to expose rare recessive variants. However, the ongoing effect of these rare alleles on modern inbred maize is unknown. To answer these questions we created a large RNAseq resource from 2200 samples collected from seven tissues across ~300 sequenced inbred lines by robotically automating a low cost ($25/sample) highly multiplexed expression profiling method. By exploiting this resource and the unique population genetics and linkage disequilibrium (LD) decay of maize, we show the outsized impact of rare alleles and evolutionary history on the regulation of expression. We consistently see that rare alleles impact the expression of all classes of genes, with the largest effects visible among the most highly expressed and network hub genes. We explicitly tie this dysregulation to fitness and show that historical bottlenecks dramatically shaped regulation as is evident from the significant (P<2.2e-16) enrichment of ancestral rare variants among eQTL. Our results reveal that even in the most intensively selected agricultural species, genetic load remains ubiquitous. This suggests that an important path to further genetic gain in artificially selected agricultural species lies in systematic purging of rare deleterious variants at the base-pair level.

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T32

Mathematical modeling predicts division plane orientation in maize cells.

(submitted by Carolyn Rasmussen <carolyn.rasmussen@ucr.edu>)
Full Author List: Martinez, Pablo[1 2]; Allsman, Lindy A.[1]; Brakke, Kenneth[3]; Hoyt, Christopher[4]; Hayes, Jordan[5]; Rasmussen, Carolyn G.[1]
[1] Department of Botany and Plant Sciences, Center for Plant Cell Biology, Institute of Integrative Genome Biology, University of California, Riverside
[2] Department of Biochemistry and Molecular Biology, University of California, Riverside
[3] Department of Mathematics, Susquehanna University
[4] Center for Plant Cell Biology NSF-REU, Harvey Mudd College
[5] Institute of Integrative Genome Biology, University of California, Riverside

Cell division is essential for the growth of any organism. One aspect of cell division that plays a critical role in proliferation and development in multicellular organisms is the location of the division axis. Proper establishment of the division axis or plane significantly contributes to the plant body organization. In order to determine how much cell geometry alone predicts division plane orientation, we used a mathematical modeling approach. Probabilistic division plane predictions of cells were made based on century-old observations of symmetric plant division: the daughter cells will have equal volume, and the dividing cell wall is a local surface area minimum. Many land plant cells form a preprophase band (PPB) composed of microtubules and other proteins that accurately predicts the future location of the new cell wall. We directly compared the geometrically predicted divisions to the location of the PPB. The PPB mostly overlapped with one of the possible types of predicted divisions. Discrepancies in PPB location compared to the predicted division occurred whSanen the neighboring cell had a cell wall or PPB directly adjacent to the division site. The PPB location shifted to avoid creating a “four-way-junction”. Avoidance of four-way-junctions, in which nearby cell edges do not align at right angles, is a long recognized structural feature in overall patterning of plant cells. Our data indicate that it reflects PPB placement deviation from the geometrically predicted division. Finally, we use the predicted divisions to determine the relative contribution of cell geometry in regulating specific division plane choices during maize leaf development. To our surprise, at a population level, the division predictions based only on cell geometry are sufficient to describe the actual divisions observed during certain stages of maize leaf development.

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

Poster Abstracts

P1

The Gene Pages at MaizeGDB

(submitted by Ethalinda Cannon <ekcannon@iastate.edu>)
Full Author List: Cannon, Ethalinda[1 2]; Belcher, Susan[3]; Gardiner, Jack[4]; Harper, Lisa[2]; Portwood, John[2]; Schaeffer, Mary[4 5]; Woodhouse, Margret[6]; Andorf, Carson[1 2]
[1] Iowa State University
[2] USDA-ARS Corn Insects and Crop Genetics Research
[3] University of Oregon
[4] University of Missouri
[5] USDA ARS
[6] University of California - Davis

Developing useful Web-based gene data pages for an organism with extensive integrated data is no small feat. At MaizeGDB we relied extensively on input from the research community to show the integration of a wide range of related data, including functional annotation, expression, proteomics, and genetic information. MaizeGDB's policy of being responsive to requests from the research community along with the existence of specific, high-value datasets that do not fit easily into generic data tables or display code, increase the complexity of building and presenting comprehensive gene pages. As well, in maize we have the additional complication that not all genetically defined loci correspond to a sequence-based gene model, and where they do correspond, that connection and its evidence has to be clear. A further challenge is that we maintain all old versions of structural annotation and are now taking in structural annotations for multiple maize genomes, where the same gene can have multiple variations. Here we describe the process of designing the gene pages, their contents, and our approaches to handling data integration.

Funding acknowledgement: United States Department of Agriculture (USDA)

P2

MaizeMine: A new data mining warehouse for MaizeGDB

(submitted by Hung Nguyen <hnn5y7@mail.missouri.edu>)
Full Author List: Nguyen, Hung N.[1]; Unni, Deepak R.[2]; Gardiner, Jack M.[2]; Le Tourneau, Justin J.[2]; Andorf, Carson M.[4]; Elsik, Christine G.[1 2 3]
[1] MU Informatics Institute, University of Missouri, Columbia MO, USA 65211
[2] Division of Animal Sciences, University of Missouri, Columbia MO, USA 65211
[3] Division of Plant Sciences, University of Missouri, Columbia MO, USA 65211
[4] USDA-ARS Corn Insects and Crop Genetics Research Unit, Iowa State University, Ames, IA 50011, USA

We are developing MaizeMine, a data mining warehouse based on the InterMine platform, to integrate genomic data for Zea mays from a variety of sources, including MaizeGDB, Gramene, Ensembl, UniProt and InterPro. MaizeMine currently contains genomic sequences and gene annotations from the B73 RefGen_v4 and RefGen_v3 assemblies, Gene Ontology, protein annotations, and protein families and domains. MaizeMine provides built-in query templates for browsing the data and flexible query building tools for creating complex queries. The Regions search tool performs queries based on lists of genome coordinates. The List tool allows users to upload identifiers to create custom datasets and perform list operations such as unions and intersections. MaizeMine is particularly useful for tracking gene identifiers across assemblies to enable meta-analyses. Query results can be downloaded in several formats (tab delimited, GFF, Fasta, BED, JSON, and XML). We are working to incorporate additional data sets, including orthologs, pathways, variation and pre-computed transcript expression values based on selected public RNAseq datasets.

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P3

Using MaizeGDB tools and interfaces to find Stocks

(submitted by Mary Schaeffer <schaefferm@missouri.edu>)
Full Author List: Schaeffer, Mary L[1 2]; Portwood, John L[1]; Sachs, Marty[3 4]; Schott, David A[1 5]; Cannon, Ethalinda K[1 5]; Gardiner, Jack M[6]; Harper, Lisa C[1]; Walsh, Jesse R[1 5]; Woodhouse, Maggie HR[1 7]; Andorf, Carson M[1 5]
[1] USDA ARS Corn Insects and Crop Genetics Research Unit; Iowa State University; Ames, IA, USA 50011
[2] University of Missouri; Division of Plant Sciences; Columbia, MO, USA 65211
[3] USDA ARS Maize Genetics Cooperation Stock Center ; University of Illinois; Urbana, IL, USA 61801
[4] University of Illinois; Department of Crop Sciences; Urbana, IL USA 61801
[5] Iowa State University; Department of Computer Science; Ames, IA, USA 50011
[6] University of Missouri; Division of Animal Sciences; Columbia, MO, USA 65211
[7] University of California Davis; Department of Plant Science; Davis, CA USA 95616

Researchers are increasingly interested in using GWAS to associated natural allelic variance with any phenotype, especially those of agronomic import. With that comes an interest in access to the germplasm used for those studies, much of which is available from USDA ARS Plant Introduction Center of Iowa (aka PI Station). Searching on accessions at GRIN-global (https://npgsweb.ars-grin.gov/gringlobal/search.aspx.) will retrieve much, but not all of the germplasm understudy. For example, mapping panels, NAM (Nested Association Mapping) and IBM (Inter-mated B73-Mo17)are currently distributed by the Maize Genetics Cooperation Stock Center (COOP) at Urbana, Illinois. Other repositories exist in other locations and will distribute small quantities of their holdings on request. Two examples include CIMMYT (International Maize and Wheat Improvement Center; http://www.cimmyt.org/) and GRIN-China (Chinese Crop Germplasm Resource Information System; http://www.cgris.net/cgris_english.html).
MaizeGDB has been integrating, over the past few years, recent trait and genotype data. While much of the curation entails careful annotation of traits with methods, references, etc, the integration hinges on properly identifying germplasm described in different studies. Where possible, MaizeGDB has linked germplasm such as that accessed in the new SNPversity tool, to a repository or developer of the germplasm, and in the case of the PI station, facilitates seed requests. This poster will provide an overview of how to find both natural and mutant germplasm at MaizeGDB using various data centers (e.g. Stock, Phenotype, Trait), the Genome Browser, and Diversity tools.

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P4

MaizeGDB: New resources for maize researchers

(submitted by John Portwood <portwood@iastate.edu>)
Full Author List: Portwood, John L[1]; Cannon, Ethalinda K[2]; Walsh, Jesse R[2]; Harper, Lisa C[3]; Woodhouse, Maggie[4]; Gardiner, Jack[5]; Schaeffer, Mary[6]; Braun, Bremen M[7]; Cho, Kyoung Tak[2]; Schott, David[2]; Sen, Taner Z[8]; Andorf, Carson M[1]
[1] USDA-ARS Corn Insects and Crop Genetics Research Unit, Iowa State University 819 Wallace Road; Ames, IA, USA 50014
[2] Iowa State University, 819 Wallace Road; Ames, IA, USA 50011
[3] USDA-ARS; 800 Buchanan Street; Albany, CA USA 94710-1105
[4] University of California - Davis; One Shields Ave.; Davis, CA, USA 95616
[5] Department of Genetics, Cellular, and Developmental Biology; 1210 Molecular Biology Building Iowa State University; Ames, IA, USA 50011
[6] USDA ARS and University of Missouri; 203 Curtis Hall; Columbia, MO, USA 65211
[7] MaizeGDB; 0031 CGIL Iowa State University; Ames, IA, USA 50011-3200
[8] USDA ARS / GrainGenes; 800 Buchanan St.; Albany, CA, USA 94710

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, integrating, and maintaining a database that serves as the central repository for the maize community. In 2009, the first publicly released maize genome reference assembly became available. At this time MaizeGDB became 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. This poster details an overview of services provided by MaizeGDB. New genome sequences are incorporated into MaizeGDB through the creation of annotation/assembly pages, BLAST databases, and a genome browser. Recent work involves genome stewardship of the B73 RefGen_v4 (Zm-B73-REFERENCE-GRAMENE-4.0) assembly, including the improvement of associations between the v3 and v4 annotations. The degree of large and complex datasets being released today has made it necessary to coordinate with different databases to facilitate greater interoperability and ease of use for our users. To productively address these issues, MaizeGDB has lead the formation of a working group, AgBioData, comprised of over 110 people from over 30 agricultural databases. New tools currently under development include MaizeMine (an InterMine instance), SNPversity (a tool for querying and visualizing SNP data), and tools for visualizing metabolic networks. Lastly, user feedback has been a valuable contribution to our success as a public service.

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P5

AgBioData: A consortium of agricultural-related databases

(submitted by Lisa Harper <lisaharper@me.com>)
Full Author List: Harper, Lisa[1]; Campbell, Jacqueline[2]; Jung, Sook[3]; Main, Dorrie[3]; Poelchau, Monica[4]; Walls, Ramona[5]; Andorf, Carson[6]; Cannon, Ethalinda[2]
[1] USDA-ARS, Albany, CA
[2] Iowa State University, Ames, IA
[3] Washington State University, Pullman, WA
[4] USDA-ARS-NAL, Beltsville, MD
[5] University of Arizona, Tucson, AZ
[6] USDA-ARS, Ames, IA

Many researchers rely on public databases for efficient access to genetic and genomic data. Indeed, the future of agricultural research depends much on the continued availability of data from publicly funded research efforts. The funding landscape of model organism databases is changing, particularly at NIH and NSF, at the same time as the volume of large data sets skyrockets. Journals seem overwhelmed, as papers can now be published without releasing the actual data that the work describes, and these data can be rapidly lost. Effective, efficient and accurate ways to manage, find and reuse huge volumes of scientific data should be a concern of all scientists. Among the agriculturally focused genomic databases, MaizeGDB has facilitated the formation of AgBioData; a consortium of agricultural biological databases which strives to manage common issues relating to data set acquisition, retrieval, display, and manipulation; to establish software, hardware and metadata standards, and to promote database best practices. AgBioData currently has about 150 members from over 25 databases. The objectives of AgBioData include coordinating with external groups such as CyVerse and scientific journals to develop standards for efficient data flow among data generators and databases; to encourage communication and sharing between databases to identify common problems and collaborative solutions; and to leverage our combined expertise to provide genomic tools for currently unsupported agriculturally important organisms. Ultimately, we aim to work together to create sustainable long term database solutions to serve the scientific community and the public.

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P6

Characterization of the surface lipid metabolic network for maize silks: Impact of genetics and environment

(submitted by Keting Chen <kchen@iastate.edu>)
Full Author List: Chen, Keting[1]; Maghoub, Umnia[2]; Loneman, Derek[2]; Peddicord, Layton[3]; Lopez, Miram[4]; Dorman, Karin[1 5]; Lauter, Nick[3 4]; Nikolau, Basil[1 3]; Yandeau-Nelson, Marna[1 2 3]
[1] Bioinformatics and Computational Biology Graduate Program; Iowa State University, Ames, IA, 50011
[2] Department of Genetics, Development and Cell Biology; Iowa State University, Ames, IA, 50011
[3] Interdepartmental Genetics and Genomics Graduate Program; Iowa State University, Ames, IA, 50011
[4] USDA-ARS Corn Insect and Crop Genetics Research Unit, Ames, IA, 50011
[5] Department of Statistics; Iowa State University, Ames, IA, 50011

The plant cuticle is comprised of cutin and is infused with and coated by long-chain non-polar and amphipathic lipids. This hydrophobic lipid layer provides protection against environmental stresses (e.g. drought, UV radiation and pathogens). The extracellular surface lipids on maize silks are comprised primarily of fatty acids and hydrocarbons, which are biochemically linked via enzymatic reactions and are the presumed precursors and end products of surface lipid biosynthesis, respectively. We have proposed a metabolic reaction network that incorporates these precursors, intermediates (i.e. aldehydes) and end-point metabolites into homologous and parallel reaction networks that occur at each chain-length of fatty acid precursor. To understand how the metabolic network is impacted by genotype, development and environment, we have characterized the surface lipid metabolome along the silk length from the two inbreds, B73 and Mo17, and their reciprocal hybrids. Using a suite of analysis methods, including principal component analysis, partial least squares discriminant analysis (PLS-DA) and Bayesian model selection, we demonstrate that both genotype and environment significantly influence the dynamics of the SL metabolome. Via PLS-DA we have identified specific lipid sets representing proposed precursor-intermediate-product relationships, which contribute substantially to the metabolomic variation observed either among genotypes, or between silks encased by husk leaves vs. those emerged into the external environment. Bayesian model selection further suggests differential impacts of genotype and environment on the presumed precursor–product ratios at different chain lengths. Collectively, these analyses provide insights into the differences in the SL metabolic network. Our future analyses of transcriptomes along the length of the silk will identify gene-surface lipid metabolite associations and will further dissect both the genetic and metabolic networks that underlie surface lipid accumulation on maize silks.

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P7

A maize gene regulatory network for phenolic metabolism

(submitted by Nan Jiang <jiang.1359@osu.edu>)
Full Author List: Jiang, Nan[1 2]; Yang, Fan[1 2]; Li, Wei[2 3]; Yu, Haidong[1 2]; Morohashi, Kengo[1 2]; Ouma, Wilberforce Zachary[1 2 4]; Morales-Mantilla, Daniel E[2 3 5]; Gomez-Cano, Fabio[1 2]; Mukundi, Eric[1 2]; Prada-Salcedo, Luis Daniel[2 3]; Velazquez, Roberto Alers[2 3 5]; Valentin, Jasmin[2 3 5]; Mejía-Guerra, Maria Katherine[1 2]; Gray, John[6]; Doseff, Andrea I[2 3]; Grotewold, Erich[1 2]
[1] Center for Applied Plant Sciences (CAPS), The Ohio State University, Columbus, OH 43210
[2] Department of Molecular Genetics, The Ohio State University, Columbus, OH 43210
[3] Department of Physiology and Cell Biology, Heart and Lung Research Institute, The Ohio State University, Columbus, OH 43210
[4] Molecular, Cellular, and Developmental Biology (MCDB) Graduate Program, The Ohio State University, Columbus, OH 43210
[5] Success in Graduate Education (SiGuE) Program, The Ohio State University, Columbus, OH 43210
[6] Department of Biological Sciences, University of Toledo, Toledo, OH 43560

The translation of the genotype into phenotype, represented for example by the expression of genes encoding enzymes, is carried out by transcription factors (TFs) that recognize specific *cis*-regulatory elements in the genes that they control. TFs and their target genes are organized in gene regulatory networks (GRNs), and uncovering GRN architecture presents an important biological challenge necessary to explain gene regulation. Linking TFs to the genes they control, central to understanding GRNs, can be performed using gene- or TF-centered approaches. We used a gene-centered approach consisting of yeast one-hybrid studies to render a network of protein-DNA interactions that participate in the transcriptional control of genes involved in the biosynthesis of maize phenolic compounds. Maize accumulates large numbers of phenolic compounds, including phenylpropanoids, lignin and flavonoids, which play important roles in plant growth and adaptation. Lignins are crucial for biomass production, and flavonoids are key nutraceuticals providing value to human and animal diets. We identified 1,100 protein-DNA interactions involving 54 phenolic gene promoters and 568 TFs. A set of 11 TFs recognized ten or more promoters, suggesting a role in coordinating pathway gene expression. The integration of the gene-centered network with information derived from TF-centered approaches provides a foundation for a phenolics GRN characterized by interlaced feed-forward loops that link developmental regulators with biosynthetic genes.

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P8

Centromeric genes of maize

(submitted by Kevin Schneider <kevinls@hawaii.edu>)
Full Author List: Schneider, Kevin L[1]; Laspisa, Daniel[1]; Presting, Gernot G[1]
[1] Molecular Biosciences and Bioengineering, University of Hawaii at Manoa, Honolulu, Hi 96822

Rearrangements of maize chromosomes since allotetraploidization placed some centromeres in the vicinity of gene-dense euchromatic regions. These formerly euchromatic regions expand significantly relative to their paralogous counterparts as a result of retrotransposon insertions. Some genes have resided at centromeres since rice/maize divergence 50 million years ago and we describe the characteristics of centromere-proximal genes.

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P9

Characterizing the diversity of brace root architecture and anatomy in maize

(submitted by Justin Sapp <jts68@duke.edu>)
Full Author List: Sapp, Justin[1]; Sparks, Erin[1]
[1] Duke University, Benfey Lab, Durham, NC, 27708

Maize brace roots, which emerge from plant stems above the soil, are proposed to play an important role in structural stability and late-stage nutrient/water acquisition. Yet how brace roots develop, integrate environmental cues and contribute to whole plant physiology remains a poorly understood area of plant biology. To quantify the diversity of brace root architecture, I obtained field-based above- and below-ground root phenotyping data from a diverse germplasm. These results show that there is vast diversity in brace root architecture. To determine the molecular networks that regulate brace root initiation and emergence, I analyzed the role of auxin in these processes. Analysis of a maize mutant that is defective in auxin transport demonstrated a defect in the number of stem nodes that produce brace roots. These results suggest that auxin plays a key role in determining the extent of brace root production in maize. These experiments are among the first to define the diversity of brace root architecture in maize, which is critical to understand the functional significance of these specialized roots.

P10

Co-expression network implicates functional divergence of gene isoforms in maize

(submitted by Dan Park <Woojun.d.park.15@dartmouth.edu>)
Full Author List: Park, Woojun D.[1]; Fu, Junjie[2]; Zeng, Erliang[3 4]; Liu, Sanzhen[1]
[1] Department of Plant Pathology, Kansas State University, Manhattan, KS. 66506, USA
[2] Institute of Crop Science, Chinese Academy of Agricultural Sciences, Beijing 100081, P.R.China
[3] Department of Biology, University of South Dakota, Vermillion, SD. 57069. USA
[4] Department of Computer Science, University of South Dakota, Vermillion, SD. 57069. USA

Gene co-expression networks are valuable for illustrating functional relationship among genes. Co-expressed genes, presumably under common expression regulations, are considered functionally associated. While gene networks have been widely explored, little has been done in generating such networks for gene isoforms. In this study, 324 public B73 RNA-Seq data sets from diverse tissues and multiple treatments were used to construct isoform co-expression networks in maize. Network analysis identified thousands of genes with isoforms occupying distinct co-expression modules, suggestive of differential regulation for the expression of sibling isoforms (isoforms from a gene). Gene ontology enrichment analysis indicated that zinc finger genes are significantly enriched in genes with differentially regulatory sibling isoforms. Network analysis also identified thousands of genes with multiple isoforms but all sibling isoforms were clustered in the same co-expression modules, implying co-regulation of sibling isoforms. This study took advantage of massive public RNA-Seq data and provided insightful information for modulation in transcriptome of maize.

P11

Computational issues in grouping complex phenotypes

(submitted by Avimanyou Vatsa <akvhxd@mail.missouri.edu>)
Full Author List: Vatsa, Avimanyou[1]; Stapleton, Ann E.[3]; Kazic, Toni[1 2]
[1] Dept. of Computer Science and Interdisciplinary Plant Group, University of Missouri - Columbia, MO
[2] Informatics Institute and Missouri Maize Center, University of Missouri - Columbia, MO
[3] Department of Biology and Marine Biology, University of North Carolina - Wilmington, NC

Discovering patterns in data is fundamental to solving the problem of providing the world's people with enough food now and in the future. A key part of ensuring food security is improving crops. Recognizing, measuring, grouping, and classifying features of crop growth, yield, and stress resistance --- some of many of the most agronomically important phenotypes --- is the first and last task in crop improvement, and at many stages in between.
Clustering is a well understood computational technique for grouping objects by a similarity criterion. So the quality of the groups constructed by clustering can only be as good as the wisdom of the computational geneticist in exploring and transforming the data, and in selecting clustering algorithms. Here, we examine the effects of common data treatments, standardization and clustering algorithms on an experimental phenotyping data set. The standardization method chosen should only minimally distort the natural clusters inherent in the data. We investigated the effects of sixteen different standardization methods on the clusters obtained by seven different clustering algorithms. We compared to a novel standardization method we call **SDFS** (*S*tandardization for *D*istribution *F*ree *S*tatistics). Our results show that clusters or groups obtained by MODECLUS using SDFS are reasonable for the experimental, orthonormal and synthetic data.

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P12

Computer vision with macroscopic microscopy: automating the extraction of pathogenesis information for quantitative analysis

(submitted by Philip Saponaro <saponaro@udel.edu>)
Full Author List: Saponaro, Philip[1 2]; Kolagunda, Abhishek[1 2]; Treible, Wayne[1 2]; Chaya, Tim[3]; Yang, Qin[4]; Wiesner-Hanks, Tyr[5 6]; Balint-Kurti, Peter[4 7]; Caplan, Jeff[3 8]; Kambhamettu, Chandra[1]; Lauter, Nick[9 10]; Nelson, Rebecca J[5 6]; Wisser, Randall J[2]
[1] Dept. of Computer and Information Sciences, University of Delaware, Newark, DE 19716
[2] Dept. of Plant and Soil Sciences, University of Delaware, Newark, DE 19716
[3] Bioimaging Center, Delaware Biotechnology Institute, Newark, DE 19716
[4] Dept. of Entomology and Plant Pathology, North Carolina State University, Raleigh, NC 27695
[5] Plant Breeding and Genetics Section, Cornell University, Ithaca, NY 14853
[6] Plant Pathology and Plant-Microbe Biology Section, Cornell University, Ithaca, NY 14853
[7] Plant Science Research Unit, U.S. Dept. of Agriculture-Agricultural Research Service, Raleigh, NC 27695
[8] Dept. of Biological Sciences, University of Delaware, Newark, DE 19716
[9] Dept. of Plant Pathology and Microbiology, Iowa State University, Ames, IA 50011
[10] Corn Insects and Crop Genetics Research, U.S. Dept. of Agriculture-Agricultural Research Service, Ames, IA 50011

The DR Maize (Disease Resistance of Maize) project uses 3D microscopy on infected tissues of contrasting genotypes to discover and validate genomic variants associated with resistance to fungal infection on maize leaves. Information captured by conventional approaches of microscopy rely on a single, small field of view and is limited in characterizing the process and features of infection. We describe work on an end-to-end computer vision system to stitch 3D confocal microscropy imagery together to form a macroscopic view while retaining microscopic detail, and to process the stitched 3D imagery to extract quantitative information about the fungal infection. Using automated computer vision modules, we extract plant host features including the leaf surface, vein locations and stomata, and fungal pathogen features including infection sites and infection network properties (depth and branching). These computer vision modules include phase correlation with global optimization, active contours, template matching, Hessian vesselness filters, and minimum spanning tree connection algorithms. The generated results are being used to quantitatively compare plant-pathogen interactions across maize genotypes, and we show results with a southern leaf blight infection. This end-to-end automated system can be used by researchers to quickly and reliably process large amounts of microscopy data for relevant properties.

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P13

Defining gene regulatory networks controlling early inflorescence development in maize

(submitted by Rajiv Parvathaneni <rparvathaneni@danforthcenter.org>)
Full Author List: Parvathaneni, Rajiv K.[1]; Shamimuzzaman, Md[1]; Kovar, Lynsey L.[2]; Vera, Daniel[3]; Bass, Hank W.[4]; Eveland, Andrea L.[1]
[1] Donald Danforth Plant Science Center, Saint Louis, MO, U.S.A, 63132
[2] NSF-REU program, DDPSC,Saint Louis, MO, U.S.A, 63132
[3] The Center for Genomics and Personalized Medicine, Florida State University, Tallahassee, FL, U.S.A, 32306
[4] Department of Biological Science, Florida State University, Tallahassee, FL, U.S.A, 32306

Inflorescence architecture is an important agronomic trait contributing to grain yield and harvestability. Numerous genes have been identified that control specific aspects of inflorescence development, however little is known about how they fit into a larger gene regulatory network to modulate architecture. Understanding the complex regulatory framework that underlies plant form will inform precise engineering and/or breeding of high-yielding crop ideotypes. Here, we integrated 38 RNA-seq datasets (both published and unpublished), that describe specific aspects of early tassel and ear development, including various mutant backgrounds and developmental stages. We used Weighted Gene Co-expression Network Analysis (WGCNA) to build a co-expression network with ~19,000 nodes and ~6.5 million edges. We also constructed ear- and tassel-specific networks. Co-expression modules that associated with key transcriptional regulators and/or marked important developmental transitions were defined. To infer upstream regulators of these co-expressed genes, we computationally mined their promoter regions for enriched cis-regulatory motifs. To narrow our search space, we integrated genome-wide chromatin accessibility maps that we generated from ear and tassel primordia. Using both a light and heavy concentration of micrococcal nuclease to digest chromatin followed by high-throughput sequencing (MNase-seq), regions of differential sensitivity were mapped. Hypersensitive sites translate to accessible chromatin and predicted transcription factor binding sites. Based on our existing ChIP-seq data for key regulators of inflorescence development, e.g. FASCIATED EAR4 (FEA4), we tested the extent to which the tissue-specific MNase data could resolve TF footprints. By integrating our chromatin accessibility maps with context-specific gene co-expression networks, we can infer transcriptional hierarchies regulating inflorescence development in maize. Further incorporating GWAS SNPs for inflorescence architecture traits revealed novel regulatory regions that we are currently testing for function.

P14

Detection of highland adaptation in maize landrace populations

(submitted by Li Wang <lilepisorus@gmail.com>)
Full Author List: Wang, Li[1]; Beissinger, Tim[2]; Roberts, Lucas[1]; Ross-Ibarra, Jeffrey[3]; Hufford, Matthew[1]
[1] Department of Ecology, Evolution and Organismal Biology, Iowa State University, Ames, IA 50011
[2] USDA-ARS, Plant Genetics Research Unit, University of Missouri, Columbia, MO 65211
[3] Department of Plant Sciences, University of California, Davis, CA 95616

Maize colonized multiple independent highland regions after originating in the Balsas River Valley in Mexico, a lowland habitat. Maize landraces from isolated highland regions exhibit convergent phenotypic characteristics, such as darker pigmentation and denser macrohairs when compared to lowland maize. Previous analysis has suggested high elevation adaptation may, in certain instances, have been obtained through chromosomal structural variation. We detected putative inversion polymorphisms through window-based principal component analysis across the genome based on the hypothesis that low recombination in the inversion regions will drive clear differentiation of haplotype groups (homozygous standard haplotypes, heterozygous haplotypes and homozygous inverted haplotypes). Maize from the Mexican, Guatemalan and southwestern US highlands was found to share inverted haplotypes present in the highland teosinte Zea mays ssp. mexicana. Further analyses confirmed that these inversions were introgressed from mexicana to specific highland populations, potentially conferring highland adaptation. We additionally identified outlier regions showing strong allele frequency differentiation between highland and lowland populations that could represent potential targets of selection during local adaptation. Significant overlap was observed for genes putatively targeted by selection in Mexican, Guatemalan and southwestern US Highlands. But limited overlap was detected for genes under selection in the Andes and in the other three highland populations. Overall, the ability to study highland adaptation in a parallel framework will enhance the general understanding of climate adaptation in maize.

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P15

Determination of gene expression in maize roots through RNA sequencing

(submitted by Alexandra Asaro <aasaro@wustl.edu>)
Full Author List: Asaro, Alexandra B[1]; Barrett, Jennifer F[1]; Baxter, Ivan[2]
[1] Donald Danforth Plant Science Center, St. Louis, MO 63132
[2] USDA-ARS, Donald Danforth Plant Science Center, St. Louis, MO 63132

Roots are the interface for plant element uptake from the soil, and thus play a significant role in shaping a plant's ionome, its full collection of mineral nutrients. Because gene regulation in the roots has a strong impact on element accumulation in the kernel, information on root gene expression can describe biological changes that cause kernel ionome variation. In this study, we examined gene regulatory networks in the maize (*Zea mays* L.) root using RNA sequencing. RNA extracts were obtained from whole roots of 218 greenhouse-grown maize lines belonging to the intermated B73 x Mo17 recombinant inbred (IBM) population, as well as the B73 and Mo17 founders. RNA sequencing produced 5.7 billion reads. To avoid mapping bias in this bi-parental population, reads were aligned to both the B73 and Mo17 reference annotations. The alignment used for gene expression quantification was chosen on a gene-by-gene basis within each recombinant inbred line according to the gene's parental origin. With these gene expression measurements, we plan to perform a genome-wide study on expression levels to identify cis-acting and trans-acting expression quantitative trait loci (eQTL).

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P16

Developing a new method for multiple species GWAS (MSG)

(submitted by Chenyong Miao <cmiao@huskers.unl.edu>)
Full Author List: Miao, Chenyong[1]; Yang, Jinliang[2]; Schnable, James C.[1]
[1] Center for Plant Science Innovation, Department of Agronomy and Horticulture, University of Nebraska-Lincoln, Linclon, NE 68588, USA
[2] Department of Agronomy, Iowa State University, Ames, IA 50011, USA

Genome-wide association (GWAS) is a powerful tool to identify the genes controlling variation in a particular phenotype. This tool has been used to identify genes associated with many agriculturally relevant traits in a wide range of species. However, GWAS does have some significant limitations. Firstly, many important genes in particular pathways may not exhibit any functional variation within a given population or species, and these genes will not be identified by GWAS analysis. Secondly, even when functional variants are present for a given gene in a given population, if one allele is present at too low a frequency, GWAS will often lack the statistical power to identify it. Seeking to address these problems, we are developing a new method called multiple species GWAS (MSG).Our ultimate goal is to apply MSG to the analysis of paired maize and sorghum data, however, the whole genome duplication in maize increases the complexity of the analysis. For our initial testing, we employed previously published SNP datasets from sorghum (*Sorghum bicolor*) and foxtail millet (*Setaria italica*). Phenotypic data was simulated across a range of heritabilities, number of QTN, effect sizes, and levels of conservation between species using 12,500 syntenic genes, ~900 accessions for each species, and ~200k SNPs for each species. MSG has the potential to increase the power to identify genes with smaller effect sizes, or where functional variants are present at low allele frequencies in one or more target species.

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P17

Developing transcriptomic resources for *Tripsacum* to study the adaptation of a maize relative to temperate climates

(submitted by Lang Yan <langyan0807@hotmail.com>)
Full Author List: Yan, Lang[1]; Lai, Xianjun[1 2]; Rodriguez, Oscar[1]; Schnable, James C.[1]
[1] Department of Agronomy and Horticulture, University of Nebraska-Lincoln, Lincoln, NE, USA 68583
[2] Maize Research Institution, Sichuan Agriculture University, Chengdu, China 611130

As the sister genus of maize, *Tripsacum* have been widely used as outgroup in studies of maize origin, evolution and domestication. Intriguingly, unlike the direct progenitor of maize, teosinte, *Tripsacum dactyloides* is native temperate climates. These climates are far more similar to those in which most commercial maize production occurs today in the United States and China. The common ancestor of the Zea/Tripsacum lineage is predicted to be adapted to tropical latitudes, so studying tripsacum is an approach to understanding how a tropical grass adapted to temperate environments through natural selection, a parallel story to how maize adapted to temperate environments through artificial selection. However, genomic resources for tripsacum, which has a large, polyploid, and high repeat content genome have been difficult to develop.
In order to improve genomic resources for tripsacum, we are conducting single-molecule sequencing of mRNAs isolated from wild *Tripsacum dactyloides* collected in eastern Nebraska. In addition to improving the utility of tripsacum as an outgroup for maize studies, this data will be used to identify genes experiencing accelerated evolution or positive in tripsacum, as well as conserved patterns of divergence in alternative splicing between homeologous genes following the whole genome duplication which is shared by both Zea and Tripsacum. Preliminary analysis with second generation sequencing data has already identified a number of genes experiencing accelerated rates of evolution in tripsacum relative to both maize and outgroup species. We hope this study can serve as a foundation for future studies aiming to take use of the wild relatives underlying the genetic basis of maize adaptation.

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P18

Dynamic transcriptome of maize ovule early development

(submitted by Gu Wei <guwei89711@foxmail.com>)
Full Author List: Wei, Gu[1]; Fei, Yi[1]; Ning, Song[1]; Xiang, Gao[1]; Jinsheng, Lai[1]
[1] State Key Lab of Agrobiotechnology and National Maize Improvement Center Department of Plant Genetics and Breeding, China Agricultural University Beijing 100193, CHINA

In maize kernel early development, ovule is the primary part of the kernel and it develops into the seed finally. Ovule contains nucellus which includes embryo sac, and integument. Taking advantage of high-through output RNA-seq, we got a set of B73 maize ovule transcriptomes that contained 31 different phases' samples. In the first 3 days after pollination, we collected the samples every 4 hours, including the non-pollinated samples, totally 19 ovule samples. In the late 3 days, we collected the samples every 6 hours, totally 12 ovule samples. In all, we detected 23,983 genes in all the phases. The lowest number was 19,351 at 80 HAP(hours after pollination), meanwhile, the highest number was 20,321 at 64 HAP. Using cluster analysis, we classified the development stages into 3 stages: stage I(0-44 HAP), stage II(48-108 HAP), stage III(114-144 HAP). Double fertilization and coenocytic were in stage I. Endosperm cellularization occurred in stage II and the cells began to differentiate in stage III. Coexpression analysis gave us an insight into the early development of ovule. Compared to the published non-seed RNA-seq data, we detected 1,084 ovule-expressed genes which contained 67 transcriptional factors. And 16 of these transcriptional factors expressed in the early phase specially and 9 transcriptional factors showed the same expression patterns. In the meantime, 321 genes were found to have the same expression patterns as 9 transcriptional factors. Through gene function enrichment analysis, these genes exhibited the enrichment in cellular process, metabolic process, cell part, cell, organelle, and organelle part patterns. This research provides a valuable resource that helps us understanding the genetic regulation of the formation of zygote, cellularizaion and cell differentiation.

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P19

European Flint reference sequences complement the maize pan-genome

(submitted by Sandra Unterseer <sandra.unterseer@tum.de>)
Full Author List: Unterseer, Sandra[1]; Seidel, Michael A.[2]; Bauer, Eva[1]; Haberer, Georg[2]; Hochholdinger, Frank[3]; Opitz, Nina[3]; Marcon, Caroline[3]; Baruch, Kobi[4]; Spannagl, Manuel[2]; Mayer, Klaus F.X.[2]; Schön, Chris-Carolin[1]
[1] Plant Breeding, TUM School of Life Sciences Weihenstephan, Technical University of Munich, Freising, Germany, 85354
[2] Plant Genome and System Biology, Helmholtz Zentrum München GmbH; Neuherberg, Germany, 85764
[3] Crop Functional Genomics, Rheinische Friedrich-Wilhelms-Universität Bonn, Bonn, Germany, 53113
[4] Energin .R Technologies 2009 Ltd (NRGENE), Ness Ziona, Israel, 7403648

The enormous diversity of maize is reflected by a large number of SNPs and substantial structural variation. To remedy the scarcity of sequence resources for the Flint pool, two reference sequences were generated *de novo* from inbred lines EP1 and F7. The two Flint lines are important founder lines of European hybrid breeding programs and trace back to the Spanish landrace Lizargarate and the French landrace Lacaune, respectively. EP1 and F7 were sequenced on an Illumina platform at 320X and 225X coverage. Using NRGene´s DeNovoMAGIC 2.0 technology, pseudochromosomes were assembled encompassing a total of 2,463 Mb and 2,405 Mb. A beta-version of the *de novo* assembled genomes, Zm-EP1-REFERENCE-TUM-1.0 (Zm00010a) and Zm-F7-REFERENCE-TUM-1.0 (Zm00011a), will be released in collaboration with NCBI and MaizeGDB prior to scientific publication in accordance with guidelines set forth by the Toronto Agreement for prepublication data sharing. Structural and functional annotation of the two genomes is currently in progress. The two reference sequences will enable integration of Flint diversity into the maize pan-genome and will pave the way for making Flint landraces amenable to crop improvement for quantitative traits.

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P20

Evaluation of dynamic growth responses of maize hybrids via, high throughput, field-based time-lapse photography

(submitted by Colton McNinch <cmcninch@iastate.edu>)
Full Author List: McNinch, Colton M[1]; Srinivasin, Srikant[1 2]; Schnable, Patrick[1]
[1] Department of Agronomy, Iowa State University; Ames, IA, 50014
[2] School of Computing and Electrical Engineering, Indian Institute of Technology Mandi; Mandi, India

During phenotyping studies traits are often only measured at a limited number of time points. Time-lapse photography enables study of plant growth rates and environmental responses at high temporal resolution. Most studies that have employed this technology have been conducted in greenhouses or other controlled environments. Unfortunately, plant performance under controlled conditions is typically poorly correlated with performance in the field. To investigate the potential value of time-lapse photography for studying plant growth and responses in agronomically relevant environments, a diverse set of maize hybrids were grown in a field site in Nebraska and imaged via time-lapse photography during most of the growing season. Trait values extracted from the time-lapse photography exhibited high correlation to ground truth (manually measured traits), suggesting the utility of time-lapse photography for field-based phenotyping.

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P21

Evolutionary genetics of natural populations of teosinte

(submitted by Anne Lorant <alorant@ucdavis.edu>)
Full Author List: Lorant, Anne[1]; Doebley, John[2]; Tenaillon, Maud[3]; Ross-Ibarra, Jeffrey[1]
[1] Univesity of California Davis; Davis, California, USA 95616
[2] University of Wisconsin; Madison, Wisconsin, USA 53706
[3] University of Paris Sud; Paris, France

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 selective sweeps 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.

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P22

Expansion of the wisconsin diversity panel to further document the maize pan-transcriptome

(submitted by Dae Kwan Ko <dkko@msu.edu>)
Full Author List: Ko, Dae Kwan[1]; Vaillancourt, Brieanne[1 2]; Hamilton, John P.[1 2]; Mazaheri, Mona[3 4]; Wang, Mei[5]; Barry, Kerrie[5]; Hirsch, Candice N.[6]; de Leon, Natalia[3 4]; Kaeppler, Shawn M.[3 4]; Buell, C. Robin[1 2]
[1] Department of Plant Biology, Michigan State University, East Lansing, MI 48824
[2] DOE Great Lakes Bioenergy Research Center, East Lansing, MI 48824
[3] Department of Agronomy, University of Wisconsin-Madison, Madison, WI 53706
[4] DOE Great Lakes Bioenergy Research Center, Madison, WI 53706
[5] Department of Energy, Joint Genome Institute, Walnut Creek, California 94598
[6] Department of Agronomy and Plant Genetics, University of Minnesota, St. Paul, MN 55108

Analysis of natural allelic variation is a useful discovery tool to identify novel alleles in genes and pathways that are consistent with agronomic productivity and environmental stability. Recent work in a number of crop species has revealed significant variation in the pan-genome in the form of copy number and presence/absence structural variants. Previous work with a transcriptome dataset derived from a set of 503 diverse inbred lines revealed 8,681 unique transcripts in the maize pan-genome/pan-transcriptome that are not present in the B73 reference genome. Sequence and transcript variants in these lines were associated with phenotypic variation of biofuel feedstock traits, suggestive that presence/absence variants in the overall maize pan-genome/pan-transcriptome may contribute to phenotypic diversity. To further our knowledgebase of genome variants that are associated with biofuel feedstock traits, we have increased our diversity datasets by expanding the Wisconsin Diversity Panel to a total of 959 inbred lines and are further characterizing presence/absence variants across this larger dataset in the context of the new B73 v4 long read genome assembly.

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P23

Fates of tandem duplications in the maize genome

(submitted by Thomas Kono <konox006@umn.edu>)
Full Author List: Kono, Thomas J.Y.[1]; Brohammer, Alex B.[1]; McGaugh, Suzanne E.[2]; Hirsch, Candice N.[1]
[1] Department of Agronomy and Plant Genetics, University of Minnesota, Saint Paul, Minnesota 55108, U.S.A.
[2] Department of Ecology, Evolution, and Behavior, University of Minnesota, Saint Paul, Minnesota 55108, U.S.A.

Gene duplication is a mutation type that has high potential to affect phenotypes. In addition to altering gene dosage, duplications may provide a substrate for further phenotypic change by allowing for rapid protein function divergence. However, not all gene duplications result in novel functional proteins; many duplicated genes lose function through pseudogenization. Here, we investigate the frequency of tandem gene duplication and the relative rates of the tandem duplicate fates in two inbred maize lines- B73 and PH207. Tandem duplicate clusters identified using the blast2raw script within the CoGe pipeline contain approximately 15% of the protein coding genes in B73 and approximately 23% of protein coding genes in PH207. Tandemly duplicated genes do not show an enrichment of transposable element sequence similarity with respect to genome wide averages when all classes of TEs are aggregated. However, LINE elements are highly over-represented in tandemly duplicated genes, and SINE elements are highly under-represented in tandemly duplicated genes, as compared to the genome wide average. We further test evolutionary rates hypotheses to compare the relative frequencies of pseudogenization, subfunctionalization, and divergent function of the tandem duplicates. This study represents the first genome-wide analysis of tandem duplicate evolution across multiple de novo genome assemblies within maize.

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P24

Field phenotype prediction on maize using novel phenomic tools and environmental information

(submitted by Zhikai Liang <zliang@huskers.unl.edu>)
Full Author List: Liang, Zhikai[1 2]; Bai, Geng[3]; Ge, Yufeng[3]; Rodriguez, Oscar[1]; Schnable, James[1 2]
[1] Department of Agronomy and Horticulture, University of Nebraska-Lincoln, Lincoln, NE, 68588
[2] Center for Plant Science Innovation, University of Nebraska-Lincoln, Lincoln, NE, 68588
[3] Department of Biological Systems Engineering, University of Nebraska-Lincoln, Lincoln, NE, 68588

Image-based phenotyping is creating new possibilities for the quantitative genetic study of crop plants. However, depending on the phenotype of interest, extracting numerical measurements from tens of thousands of plant images remains challenging, requiring the development and testing of new computer vision algorithms. Early efforts in this area have focused on replicating transitional phenotypes which have been manually measured under field conditions in the past. However, image-based phenotyping also creates the possibility to identify and study the genetic basis of variation novel phenotypes which would not be practical to measure manually, but which may be linked to agronomic performance under field conditions. In order to stimulate the development of new algorithms for extracting novel quantitative measurements of plant architectural and compositional phenotypes we have generated a set of controlled environment phenotypic data for maize lines where are also being grown and phenotyped at field locations across the US as part of the public-private Genomes to Fields (G2F) project. The first, already released dataset consists of RGB, thermal infrared, and hyperspectral data from each plant collected once per day during vegetative development (available at http://plantvision.unl.edu/dataset). A second soon to be released dataset will include data from 139 maize hybrids and extend image capture through flowering. Currently we are able to extract only crude measurements from the resulting image datasets, but these data can already produce large numbers of synthetic and derived phenotypic traits, suggesting that dimensional reduction and trait selection will both be important initial steps in any attempt to identify meaningful correlations between architectural and compositional traits in the greenhouse and field performance under different environmental conditions.

P25

Functional characteristics of co-expression gene islands in maize genome

(submitted by Cheng Huang <hc66@cau.edu.cn>)
Full Author List: Huang, Cheng[1]; Wang, Xufeng[1]; Chen, Qiuyue[1]; Wu, Yaoyao[1]; Tian, Feng[1]
[1] National Maize Improvement Center of China; China Agricultural University; No. 2, Yuanmingyuan west Road, Beijing, China, 100193

Maize provides a perfect experimental model to study the characteristics of the genome because of its complete genome sequence, gene annotation and increasing expression data. Here, through investigating the expression similarity of neighboring genes, we presented the identification and analysis of local co-expression clusters (so-called co-expressed gene islands) in the maize genome. We employed the public expression data set that covers different developmental stages and tissues from the qTeller website and the recently published data set from a specific seed cell analysis (Chen et al. 2013). With these expression data, we identified 1,502 and 2,415 co-expressed gene islands, respectively, which are significantly higher than to be expected by chance alone. These co-expression islands or clusters spread through the whole genome, not showing any chromosomal position effects. Most of gene islands are miniature, residing with two co-expressed gene members, and the larger gene islands are relatively rare. As expected, tandemly duplicated genes can favor the occurrence of co-expression gene islands. We did not found the evidence that shared promoter sequence is one of the major reasons for such chromosomal islands in maize genome. Gene distance, to some degree, could explain the existence of highly co-expressed gene pairs or clusters, which is consistent with the famous ripple effects in some eukaryotic genomes. However, there are still some co-expressed genes clusters which might cause by other parameters, e.g. sharing similar regulatory elements and chromatin environment or co-function. Although no apparent tissue specificity was observed, we revealed that some gene clusters showed a certain degree of expression preference. The biggest gene cluster, consisting of six genes, tends to be higher expressed in silk, ovule and the early stage of seeds.

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P26

G4 quadruplexes in and near regulatory elements of maize genes involved in tissue development and altered transcriptional response to abiotic stresses

(submitted by Mingze He <mhe@iastate.edu>)
Full Author List: He, Mingze[1 2]; Andorf, Carson[3 4]; Walley, Justin W.[1 5 6]; Walia, Harkamal[7]; Koch, Karen[8]; Liu, Peng[1 9]; Bass, Hank W.[10]; Lawrence-Dill, Carolyn J.[1 2 6 11]
[1] Bioinformatics and Computational Biology Program, Iowa State University, Ames, Iowa, USA, 50011
[2] Department of Genetics, Development and Cell Biology, Iowa State University, Ames, Iowa, USA 50011
[3] USDA-ARS Corn Insects and Crop Genetics Research Unit, Iowa State University, Ames, Iowa, USA 50011
[4] Department of Computer Science, Iowa State University, Ames, Iowa, USA 50011
[5] Department of Plant Pathology & Microbiology, Iowa State University, Ames, Iowa, USA, 50011
[6] Genetics and Genomics program, Iowa State University, Ames, Iowa, USA, 50011
[7] Department of Agronomy and Horticulture, University of Nebraska-Lincoln, Lincoln, Nebraska, USA, 68588
[8] Plant Molecular and Cellular Biology Program, Horticultural Sciences Department, Genetics Institute, University of Florida, Gainesville, FL 32611, USA
[9] Department of Statistics, Iowa State University, Ames, Iowa, USA 50011
[10] Department of Biological Science, Florida State University, Tallahassee, FL, USA 32306-4295
[11] Department of Agronomy, Iowa State University, Ames, Iowa, USA 50011

G-quadruplexes (G4s) are reversible three-dimensional DNA structures that occur throughout genomes. G4 DNA motifs can be found using computational sequence pattern searches, such as those for the classical single-stranded G4 motif d(G3+N1-7G3+N1-7G3+N1-7G3+). In maize, G4 motifs occupy non-random sites including antisense (i.e., template) 5' UTR hotspots of genes associated with low energy signaling and responses (i.e., hypoxia, low sugar, and nutrient deprivation; Andorf et al., 2014 J Genet Genomics). We examined transcriptome data from stress-response studies in order to test for indirect functional evidence of their contribution to gene regulation. Genes with G4s in or near regulatory regions respond strongly to diverse stress conditions including submergence, cold, heat UV, salt, and cold stress. As a group, they are nearly twice as likely as non-G4 genes to increase expression in response to stress. GO enrichment studies indicate that G4 genes were enriched in specific TF families (i.e., NAC, HSF, CCAAT-HAP2, and ZIM), and that differentially expressed G4-containing genes are likely to be involved in "developmental processes", suggesting that altered growth rates may be a specific component of the stress response. Co-expression network analyses revealed that G4 motifs are strongly associated with stress activation of transcription factors at hub positions in the network. In addition, transcriptomic and proteomic analyses across 55 tissues and developmental stages in non-stress conditions revealed tissue specific patterns of expression. Our results add new evidence to the idea that G4s confer stress-response and gene regulatory properties to genes in maize, possibly acting at transcriptional and translational control levels in stress and development.

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P27

Genomaize, a UCSC genome browser for maize genomes

(submitted by Daniel Vera <vera@genomics.fsu.edu>)
Full Author List: Vera, Daniel[1]; Kyle, Kathleen[1]; Bass, Hank[2]
[1] The Center for Genomics and Personalized Medicine; The Florida State University; Tallahassee, FL, 32306
[2] The Department of Biological Science; The Florida State University; Tallahassee, FL, 32306

Visualization is a critical process for the assessment and interpretation of genomic data. The UCSC Genome Browser is a web-based platform that offers a rapid, intuitive, and centralized means of viewing various types of genomic data mapped to reference genomes. Despite its great utility, the browser hosted at UCSC does not host plant genomes. To address this issue, we have established genomaize, a UCSC genome browser specifically for maize genomes. Genomaize currently hosts B73 and W22 genomes, and will also host newly sequenced maize genomes as they become publicly available. Users may upload and visualize custom private tracks, save sessions that can be shared with other users, perform BLAT alignments against reference maize genomes, and perform additional functions built into the browser. We also provide the software "gtracks" (https://github.com/FSUgenomics/gtracks) for easily maintaining large and stable catalogs of private tracks in the UCSC genome browser using google spreadsheets. Genomaize is accessible at http://genomaize.org.

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P28

Genome assembly of maize Ki11 genome using long-read sequencing

(submitted by Bo Wang <bwang@cshl.edu>)
Full Author List: Wang, Bo[1]; Regulski, Michael[1]; Church, Deanna[2]; Hastie, Alex[3]; Goodwin, Sara[1]; McCombie, W. Richard[1]; Ware, Doreen[1 4]
[1] Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724
[2] 10x Genomics Inc. 7068 Koll Center Parkway, Pleasanton, CA 94566
[3] BioNano Genomics, San Diego, CA 92121
[4] USDA ARS NEA Robert W. Holley Center for Agriculture and Health Cornell University, Ithaca, NY 14853

Maize is a very good model for diversity investigation, and more and more studies have revealed that structural variation widely exists among different maize lines, which contribute to phenotypic diversity. However, increasing evidence has shown that just one single reference genome sequence is not enough to exploit the genomic diversity character in this species. Recently, we generated a brand new version of the B73 maize reference genome sequence using single-molecule sequencing. In the meantime, there are several other maize genomes that have been generated using short-read sequencing approaches (e.g. PH207, W22). However, comparison of different genomes generated using different approaches can confound reliable results, considering the quality of short-read assemblies is far from that of the long-read sequencing approach. Here, we are sequencing a tropical maize line named Ki11 using PacBio single-molecule sequencing. The PacBio RSII platform yielded ~60x genome coverage data, which is similar to maize B73 PacBio sequencing. In addition, 10x Genomics sequencing was applied for the assembly as well, which generated ~56x genome coverage data. And previously, we generated a BioNano genome map of Ki11 using the Irys system. Combining these three methods, we are able to generate a comparable high quality genome sequence of the Ki11 line, which will enable a detailed look between different maize lines for many biological studies, especially the maize diversity research, as well as heterosis study.

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P29

Global gene expression analysis of a dosage series of chromosome arm 1L

(submitted by Adam Johnson <afj8c8@mail.missouri.edu>)
Full Author List: Johnson, Adam F[1]; Hou, Jie[2]; Islam, Soliman[2]; Cheng, Jianlin[2]; Birchler, James A[1]
[1] Division of Biological Sciences, University of Missouri, Columbia, MO 65211
[2] Department of Computer Science, University of Missouri, Columbia, MO 65211

Previous results in maize on the individual gene level had indicated the modulation of genes across the genome when only a portion of the genome was varied in dosage. To examine this effect on a global level an RNA-Seq experiment was conducted for a dosage series of chromosome arm 1L. The dosage series can be generated using the B-A translocation, TB-1La. Diploid plants with 2, 3, and 4 copies of 1L were compared to each other in terms of gene expression as measured via RNA-Seq. Further comparisons were made with haploid plants containing 1 and 2 copies of 1L genes. Genes in cis on 1L have expression ratios that range from a dosage effect, ~1.50, to near dosage compensation. Genes not on 1L are in trans and can be unaffected or reduced to varying levels, primarily to the inverse of the dosage imbalance. Interestingly, the distribution of gene modulations comparing 1 to 2 copies in a haploid was somewhat distinct from comparing 2 to 4 copies in a diploid despite the same level of genomic imbalance. Research supported by NSF grant IOS-1545780.

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P30

GRASSIUS: Helping unravel the regulatory repertoire of the grasses

(submitted by Wilberforce Ouma <wilberzach@gmail.com>)
Full Author List: Ouma, Wilberforce Z[1 2]; Maina, Eric M[2 4]; Gomez Cano, Fabio A[2 4]; Doseff, Andrea I[3 4]; Gray, John[5]; Grotewold, Erich[2 4]
[1] Molecular Cellular and Developmental Biology Graduate Program, The Ohio State University, Columbus, Ohio, USA, 43210
[2] Center for Applied Plant Sciences, The Ohio State University, Columbus, Ohio, USA, 43210
[3] Department of Physiology and Cell Biology, Dorothy M. Davis Heart and Lung Research Institute, The Ohio State University, Columbus, Ohio , USA, 43210
[4] Department of Molecular Genetics, The Ohio State University, Columbus, Ohio, USA, 43210
[5] Department of Biological Sciences, University of Toledo, Toledo, Ohio, USA

Understanding control of gene expression in crop plants is vital for efforts aimed at increasing crop productivity and improved agronomic traits such as stress tolerance. Apart from its role as a major cereal, maize provides an attractive system for investigating the architecture of gene regulatory networks (GRNs) and the underlying gene regulatory grids (GRGs) in cereal crops. We have developed GRASSIUS (grassius.org) as a gene regulatory information knowledgebase for the grasses. GRASSIUS hosts regulatory databases as well as computational and experimental resources that aid in understanding control of gene expression in the grasses. GRASSIUS consists of three interlinked databases that contain a collection of transcription factors (TFs) classified into different families (GrassTFDB); transcriptional co-regulators (GrassCoRegDB); and promoter sequences (GrassPROMDB) for maize and other grasses including rice, sorghum, sugarcane, Brachypodium and Setaria. In addition, GRASSIUS hosts experimentally determined TF/coregulator protein-DNA interactions (PDIs) that can be visualized as regulatory networks using the GRASSIUS Regulatory Grid Explorer (GRG-X). Included in GRASSIUS is a collection of approximately 2100 maize TF ORFs (TFome collection); newly annotated maize transcription start sites (TSSs) derived from Cap Analysis of Gene Expression (CAGE) experiments; and analytical tools such as GRASSIUS Blast, Perl modules and custom-made scripts for generating visual representations of protein domains, gene structures and cis-regulatory elements. The utility of GRASSIUS to the scientific community therefore provides opportunity for accelerated discovery and elucidation of regulatory mechanisms that are vital for engineering cereal crops with improved agronomic traits.

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P31

High throughput automatic mosaicing of the field from drone aerial imagery

(submitted by Rumana Aktar <rayy7@mail.missouri.edu>)
Full Author List: Aktar, Rumana[1]; Aliakbarpour, Hadi[1]; Bunyak, Filiz[1]; Kazic, Toni[1]; Seetharaman, Guna[2]; Palaniappan, Kannappan[1]
[1] Dept of Computer Science, University of Missouri, Columbia, MO, USA
[2] US Naval Research Laboratory, Washington D.C., USA

Aerial imagery of field-grown crops has enormous potential to measure complex, agronomically important phenotypes. A single flight images the same field from many different camera angles, vehicle altitudes, and vantage points; and video imagery captures the plants' movement and growth. The first step in phenotypic analysis is to mosaic the imagery so that the same features are in registration throughout the video sequence, despite changes in vehicle and camera position and plant movement.
The volume of data collected and the similarities among and complexity of the features in video imagery make generating a mosaic very challenging. Many computational methods rely heavily on the presence of distinct features in the imagery, but these occur very infrequently and sporadically in the field. Moreover, manual placement and maintenance of such features is labor-intensive. Traditional approaches to mosaicing are computationally intensive: the time required scales quadratically with the number of frames in the video. We have developed a linear-time approach where only consecutive frames are matched. We also introduce a fused descriptor, a combination of the structure tensor and the normalized cross correlation, which ensures that we get a time-efficient and robust matching between consecutive frames. Finally, we use ASIFT to guarantee excellent matching between two distant frames. Our experiments show the efficiency and precision of the proposed method for automatic image mosaicing of crop fields.

P32

High-throughput phenotyping detects large natural variation of maize growth at seedling and early juvenile stages, and its correlation with adult phenotypes

(submitted by Abdalla Zanouny <azibrahim@wisc.edu>)
Full Author List: Zanouny, Abdalla I.[1]; Kaeppler, Shawn M.[1]
[1] University of Wisconsin-Madison, 1575 Linden Dr, Madison, Wisconsin, USA, 53706

Early seedling vigor and juvenile vegetative growth are important to determining final yield allowing plants to establish and access nutrients and water, providing competition against weeds, and allowing mechanical cultivation in production systems that do not use herbicides. We utilized digital imaging to track the growth of 3036 plants representing 449 diverse maize lines 13 through 32 days after planting. From the 123,000 acquired images, 137 traits were extracted that relate to plant biomass and architecture. Digital Biomass, a measure of plant vigor, was estimated from the top and side images and strongly correlated with plant shoot weight at harvest. Maximum genotypic variability for Digital Biomass was found at day 13. Vigorous genotypes consumed more water reflecting their ability to take advantage of available resources. Several image-based traits showed a significant change over time that reflected plant growth. The incorporation of these traits may improve the accuracy of models predicting plant biomass.
The value of image-based young plant traits was evaluated as a predictive tool for adult phenotypes measured in three field seasons. Weak to moderate correlations were obtained for traits such as flowering date, growing degree days (GDD), ear height, and plant height. This study highlights two advantages of screening germplasm at early seedling stage: First, the maximum genetic variability for Digital Biomass during the first third of the maize life cycle was found to be at the seedling stage (day 13), selection during this stage this stage may allow more gain from selection. Prediction of flowering based on genomic data might be improved when complemented with phenotypic screening at the seedling stage, especially in earlier stages of a breeding program where a large number of genotypes are evaluated. This study also suggests testing the utility of other image-based phenotypes in predicting plant biomass.

P33

Highly-interwoven communities of gene regulatory network unveil crucial genes for maize seed development

(submitted by Wenwei Xiong <xiongwe@mail.montclair.edu>)
Full Author List: Xiong, Wenwei[1]; Wang, Chunlei[2]; Zhang, Xiangbo[2]; Yang, Qinghua[3]; Shao, Ruixin[3]; Lai, Jinsheng[2]; Du, Chunguang[1]
[1] Department of Biology, Montclair State University, Montclair, NJ 07043
[2] National Maize Improvement Center, China Agricultural University, Beijing 100083, China
[3] National Key Laboratory of Wheat and Maize Crop Science, Henan Agricultural University, Zhengzhou 450002, China

The complex interactions between transcription factors (TFs) and their target genes essentially govern the dynamic process of maize seed development. Topological properties of genes in the regulatory network often reflect the importance of their roles and contributions. Identifying the most important players in maize seed development can have profound implications in our understanding of the genetic control as well as agriculture. Here we aim to achieve this goal through a data mining approach that integrates spatial-temporal RNA-Seq profiles, microdissected compartments data of maize seed, and manually curate KEGG pathways. First, we reverse engineered a regulatory network from genes as nodes and their interactions as edges based on information theory. Then we studied collective gene interaction patterns and uncovered highly-interwoven network communities as the building blocks of the regulatory network. We propose that communities with the highest connectivity and scores are crucial to sustain the network integrity. Here we discovered two such high-profile communities. One consists of mostly unknown genes interacting with well-studied *bt1, o2* and *shrunken2*. Their tight interactions may contribute to various kernel phenotypes. Second community has a surprising 83% of genes located in the basal endosperm transfer layer. Furthermore, we found that top highly-connected network hubs tend to collaborate. We also provides 73 new candidate genes as direct targets of *o2*, among many other known/unknown TFs. We identified non-TF genes with high collective-influence, and found that they are mostly regulated by hub TFs, e.g. two top unknown genes from the conducting zone share regulators with the *zein* genes. To ensure our network reliability, we further verified a subset of predicted TF-gene bindings with our yeast one hybrid assays as well as published ChIP-Seq data. In conclusion, this study uncovers genes and functional units as communities working collaboratively that are crucial to maize seed development.

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P34

Hybridization between parapatric teosinte populations results in the formation of three unique hybrid groups in Mexico

(submitted by David Hufnagel <davidehuf@gmail.com>)
Full Author List: Hufnagel, David E.[1]; Kananen, Kathryn[1]; Doebley, John F.[2]; Glaubitz, Jeff C.[3]; Gonzalez, Jesus S.[4]; Hufford, Matthew B.[1]
[1] 339A Bessey Hall, Iowa State University, Ames, IA, USA 50014
[2] Laboratory of Genetics, University of Wisconsin, Madison, Wisconsin, USA 53076
[3] 175 Biotechnology Building, Ithaca, NY, USA 14853
[4] Km. 15.5 Carretera Guadalajara-Nogales, Las Agujas, Zapopan, Jalisco. C.P. Mexico 44171

Hybridization is a largely underappreciated force in evolution. Hybridization can improve adaptability through new allelic combinations and can facilitate speciation especially when hybrids are isolated from their progenitors. The closely related, parapatric teosinte (i.e., wild maize) subspecies Zea mays ssp. parviglumis (parviglumis) and Zea mays ssp. mexicana (mexicana) diverged recently, yet they both show clear signs of local adaption as well as ongoing hybridization in putative hybrid zones. Parviglumis inhabits the Pacific coastal lowlands and mexicana inhabits the highlands of Mexico with some degree of range overlap at intermediate elevations. Using a broadly sampled, but low-density genomic SNP dataset and population genetic methods we have identified three groups of parviglumis-mexicana hybrids in hybrid zones: one in the Central Plateau of Mexico and two in the Balsas River Basin of Mexico. We found that these hybrid groups are genotypically distinct and have unequal genetic similarity to each parental subspecies. However, all hybrid populations show similarity to a single race of both parviglumis and mexicana suggesting a common ancestor for these hybrid groups.

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P35

Identification and characterization of distant enhancers in *Zea mays*

(submitted by Blaise Weber <b.b.m.weber@uva.nl>)
Full Author List: Weber, Blaise[1]; Oka, Rurika[1]; Zicola, Johan[2]; Wesselink, Jan-Jaap[3]; Hoefsloot, Huub[1]; Turck, Franziska[2]; Stam, Maike[1]
[1] University of Amsterdam, Science Park 904, 1098XH Amsterdam, The Netherlands
[2] Max Planck Institute for Plant Breeding, Carl-von-Linné 10, 50829 Cologne, Germany
[3] Diagenode, S.A, Rue du Bois Saint-Jean 3, 4102 Liège, Belgium

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 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 (eRNAs) 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*. Putative regulatory sequences are being identified using published bisufite-seq data sets and newly generated DNAseI-seq, ChIP-seq, RNA-seq and CAGE-seq data sets in two different tissues: the inner stem of young V2 seedlings and husk leaves. Our data indicate the existence of about 1400 putative distal enhancers, which also include known and experimentally validated enhancers in maize. The enhancer candidates are characterized by increased chromatin accessibility, low DNA-methylation levels and a preferential enrichment of H3K9ac on one side of the DHS peak. Selected candidate sequences are currently being validated using in planta reporter systems. Furthermore, RNA-seq data are used to predict the target genes of the candidate enhancers, and for a limited number of candidates this prediction is currently being tested with 4C (Circular Chromosome Conformation Capture).

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P36

Identification of inversion polymorphisms from genomic data using a nonparametric Bayesian estimator

(submitted by Luis M. Avila <lavila@ucdavis.edu>)
Full Author List: Avila, Luis M.[1]; Mei, Wenbin[1]; May, Michael R.[2]; Ross-Ibarra, Jeffrey[1 3]
[1] Department of Plant Sciences, University of California, Davis, CA 95616, USA
[2] Center for Population Biology and Department of Ecology and Evolutionary Biology, University of California, Davis, CA 95616, USA
[3] Center for Population Biology and the Genome Center, University of California, Davis, CA 95616, USA

Inversion polymorphisms have been associated with gene shuffling, suppression of chromosomal crossovers, adaptation and fitness in many organisms. Their identification can thus provide insight into the evolutionary history of populations and provide additional resources for crop improvement. Initially identified from cytological observations, methods capable of identifying inversions from genotypic data make it possible to leverage existing genotyped populations for the identification of novel inversions and the study of their segregation and association with phenotypes. When analyzing densely genotyped populations inversions can be identified in regions of increased linkage disequilibrium (LD). Alternatively, dimensionality reduction methods such as principal component analysis (PCA) or multidimensional scaling (MDS) can be applied to genotypic data to identify inversions, producing distinctive clustering patterns around inversion breakpoints. We have developed a Bayesian non-parametric method for identifying inversions using individual components from dimensionality reduction methods applied to genotype data. We are making this tool available as an R package, with speed optimizations and easy to follow documentation for other researchers to perform genomic scans for inversions.

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P37

Identifying indicators of cold tolerance in maize root exudate through NMR fingerprinting

(submitted by Connor Long <connor.long@doane.edu>)
Full Author List: Long, Connor[1]; Meier, Nate[1]; Garver, Billy[1]; Oyebefun, Josiah[1]; Long, Alaetra[1]; Okuom, Macduff[1]; Stolze, Nick[1]; Nieveen, Jenna[1]; Doan, Tu[1]; Swancutt, Rhiann[1]; Durham Brooks, Tessa[1]
[1] Doane University, Crete, NE, 68333

Expanding the duration of the growing season in maize is one way to improve yields. However, early planting exposes seedlings to cold stress, which could compromise adult growth. Therefore, mechanisms of cold tolerance are important potential targets for crop improvement. Identification of early biomarkers that could reliably predict the impact of seedling cold stress on adult growth would make it possible to more efficiently identify genetic factors that contribute to cold resistance. The objective of this study was to determine the feasibility of seedling root exudate composition after cold stress as a biomarker. Root exudate was collected from 3 day old seedlings of twelve Maize genotypes in control and cold stress conditions. Seedlings were removed from their growth tubes and transferred to a plot on Doane University's campus. Weekly height measurements (cm) were taken for each seedling, as was shoot and root biomass after the growing season had concluded. Root exudate fingerprints were collected using a previously established technique from one cold tolerant (B73), one cold susceptible (B97), and two canalized (CML103 and CML247) genotypes using Nuclear Magnetic Resonance (NMR) spectroscopy. Analysis methods for investigating differences in overall fingerprint composition and relative concentrations of sugars and amino acids between genotype and conditions is underway.

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P38

Improving maize centromere sequences of RefGen V4

(submitted by Daniel Laspisa <dlaspisa@hawaii.edu>)
Full Author List: Laspisa, Daniel J[1]; Schneider, Kevin L[1]; Wolfgruber, Thomas K[1]; Jiao, Yinping[2]; Ware, Doreen[2]; Maize B73, AGPv4 Consortium[2]; Presting, Gernot G[1]
[1] Molecular Biosciences and Bioengineering, University of Hawaii at Manoa, Honolulu, Hi 96822
[2] Cold Spring Harbor Laboratory, Cold Spring Harbor NY 11724; USDA-ARS, PSNR, Ithaca, NY, 14853

Maize centromeres are marked epigenetically by the centromere-specific histone variant CENH3 and are composed primarily of Centromeric Retrotransposons (CR) and the tandem repeat CentC. Correct assembly of the maize centromeres is essential for reconstructing their evolution, but these repeat-rich regions are difficult to assemble and remain fragmented or incomplete even in the best reference genome available today, the Zea mays B73 Refgen V4 assembly constructed from long reads from Single Molecule Real-Time sequencing technology (PacBio) and NanoChannel Array from BioNano. We manually edited over 84 Mb of maize centromere sequence spanning eight of the ten centromeres, closed over 75 sequence gaps, added 5 megabases of DNA sequence to the assembly, and corrected chimeric sequences in both high and low quality unitigs. For centromere 5, we were able to confirm the recent insertion of >50 complete CR2 elements predicted from junction data in an earlier version of the reference genome that was not well assembled across repeat regions. We plan to manually assembly the final two centromeres, perform a final correction of all centromere regions using the 60X coverage reads generated by the Ware laboratory, and submit all sequences using the Genome Reference Consortium (GRC) tools from NCBI to update the reference genome. The goal is to close all sequence gaps flanked by <10 kb of tandem repeat.

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P39

Integrating co-expression networks with GWAS to detect causal genes driving elemental accumulation in maize

(submitted by Robert Schaefer <schae234@umn.edu>)
Full Author List: Schaefer, Robert J[1 2]; Michno, Jean-Michel[2]; Jeffers, Joseph[5]; Dilkes, Brian[3]; Baxter, Ivan[4]; Myers, Chad L[2 5]
[1] Department of Veterinary Population Medicine, University of Minnesota, St Paul, MN 55108
[2] Bioinformatics and Computational Biology Graduate Program, University of Minnesota, Minneapolis, MN 55455
[3] Department of Biochemistry, Purdue University, West Lafayette, IN 47907
[4] USDA-ARS, Donald Danforth Plant Science Center, St. Louis, MO 63132
[5] Department of Computer Science, University of Minnesota, Minneapolis, MN, 55455

Genome wide association studies (GWAS) have identified thousands of loci linked to hundreds of traits in many different species. However, in many cases, the causal genes and and the cellular processes they contribute to, remain unknown. This problem is even more pronounced in non-model species where functional annotations are sparse and there is poor resolution in single nucleotide polymorphism (SNP) boundaries. The vast amounts of data available from high throughput sequencing, such as RNA-Seq, are a tantalizing resource to leverage in identifying potential candidates under GWAS SNPs, though are often underutilized or difficult to interpret. To mitigate these issues, here, we systematically integrate whole genome SNP data with functional information derived from gene co-expression networks using a computational framework called Camoco.
Camoco scores interactions among genes near GWAS peaks and establishes significance using a robust bootstrapping model. We demonstrate the precision of our method by simulating GWA studies using Gene Ontology (GO) terms. We then used our method to functionally inter-relate loci identified in a large scale, GWA study characterizing elemental accumulation in maize kernels. Our results demonstrate that simply taking the closest genes to significant GWAS SNPs will often lead to spurious results demonstrating the need for proper functional modeling and bootstrapping. Additionally, when deriving functional information from gene transcriptional networks, the biological context from which the transcription was measured is essential. Inclusion of gene expression data from tissues not relevant to the elemental phenotypes collected abolishes the relationships between the co-expression networks and the GWAS SNPs. In the correct biological context, genes linked to GWAS hits for elemental accumulation were more significantly co-expressed than genes within similarly structured GO terms. Our framework provides a method to systematically evaluate the putative functional relationships among GWAS candidate loci as well as to efficiently prioritize gene lists produced from GWA studies.

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P40

KBCommons: A multi 'OMICS' integrative framework for database and informatics tools

(submitted by Shuai Zeng <zengs@mail.missouri.edu>)
Full Author List: Zeng, Shuai[1 2]; Joshi, Trupti[1 2 3 4]
[1] Department of Computer Science
[2] Christopher S. Bond Life Sciences Center
[3] MU Informatics Institute
[4] Department of Molecular Microbiology and Immunology and Office of Research, School of Medicine

Advancement of next generation sequencing and high-throughput technologies has resulted in generation of multi-level of 'OMICS' data for many organisms. However, these data are often individually scattered across different repositories based on data type, making it difficult to integrate them. We have addressed this issue through our in-house developed Soybean Knowledge Base (SoyKB) framework, a comprehensive web-based resource that bridges translational genomics and molecular breeding research in soybean. It acts as a centralized repository for soybean multi-omics data, and is equipped with an array of bioinformatics analytical and graphical visualization tools. It is available at http://soykb.org and has proven to be a great success with more than 500 registered users.
Users working on other biological organisms including plants, animals and biomedical diseases have similar needs and the developed framework can be easily expanded to make the visualization and analysis tools function for other organisms, without having to reinvent the wheel. To achieve this we have developed KBCommons, a platform that automates the process of establishing the database and making the tools for other organisms available via a dedicated web resource. It provides information for six entities including genes/proteins, microRNAs/sRNAs, metabolites, SNP, traits as well as plant introduction or strains/populations. It also incorporates several multi-omics datasets including transcriptomics, proteomics, metabolomics, epigenomics, molecular breeding and other types. We have currently expanded KBCommons framework and tools to *Zea mays , Arabidopsis, Mus musculus* and *Homo sapiens*.
We have integrated various genomics dataset for maize including RNAseq B73 mutants and Tassel meristem from our collaborators. It provides a suite of tools such as the gene/metabolite pathway viewer, Protein Bio-Viewer, heatmaps, scatter plots and hierarchical clustering. It also provides access to PGen, Pegasus analytics workflows developed for genomics variations analysis. It also has suite of tools for differential expression analysis of transcriptomics and other multi-omics datasets including venn diagrams, volcano plots, function enrichment and gene modules.

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P41

Landmark-based semi-automated phenotyping for developmental phenotypes

(submitted by John Hodge <jgerardhodge@gmail.com>)
Full Author List: Hodge, John G[1]; Li, Qing[1]; Doust, Andrew N[1]
[1] Department of Plant Biology, Ecology, and Evolution, Oklahoma State University. 301 Physical Sciences, Stillwater, OK 74078

Phenotyping plants across their lifecycle can provide key insights into how their morphology at maturity is regulated by developmental mechanisms. This can especially be the case for labile traits that may shift either in response to physiological stress or perceived changes in the environment. With this issue in mind we have built a pipeline using time series image data for quantifying patterns of growth based on pseudolandmarks. Our method for identifying and extracting pseudolandmarks in time series data has broad applications for measuring plant stature and architecture between different genotypes, including mutants that may have subtle developmental effects. It will also be useful for characterizing large crossing populations. We have tested the pipeline on a set of recombininant inbred lines selected from a *Setaria italica* (foxtail millet) X *S. viridis* (green foxtail) mapping population, particularly focusing on pseudolandmarks associated with axillary branch outgrowth and elongation. This method has allowed us to characterize where deviations in plant stature occurs between lines either due to differences in lateral branch outgrowth and elongation of the main culm with greater temporal resolution and less subjectivity than would be possible with manual measurements. Our method of high throughput phenotyping opens up possibilities for identifying subtle variations within populations that could otherwise be missed.

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P42

Machine learning approaches for data integration to model regulatory architectures

(submitted by Maria Mejia Guerra <mm2842@cornell.edu>)
Full Author List: Mejia-Guerra, Maria K[1]; Zuo, Tao[1]; Buckler, Edward S[1 2]
[1] Institute for Genomic Diversity, Cornell University, Ithaca, New York, United States of America
[2] United States Department of Agriculture/Agricultural Research Service, Ithaca, New York, United States of America

The rewiring of regulatory networks from which phenotypic variation may arise is the result of the turnover of *trans* specificity and *cis* regulatory elements within enhancers and promoters. An emerging view of the regulatory architecture suggests that recognition of the *cis*-information by *trans* regulators involves a combined readout of **DNA structural features, DNA sequence motifs, motif spatial organization, and the underlying sequence and chromatin context in which motifs are embedded.**
Here, we present a supervised machine learning approach to identify kmer patterns in regulatory regions by a flexible model that integrates raw sequence and sequence variation constraints. We tested our approach to identify functional lexicons and grammars from experimentally determined *in vivo* transcription factor binding locations, core promoters and enhancer data in maize. The approach here presented has as a goal the discrimination of functional categories from equivalent sequences randomly sampled from the genome, maintaining chromosomal and GC% distribution. The model performance was evaluated with the area under the receiver operating characteristic curve (AUROC), for which 1 is the highest possible score for a perfect classification. Overall, our proposed model achieved ~0.80 for transcription factors, ~0.90 for core promoters and ~0.85 for enhancer data.
The model generates regulatory scores that can be used to predict the potential effect of genetic variants on the overall regulatory region architecture. In a broad perspective, our approach provides a quantitative model of the relationship between different layers of information placed on the genome for several non-coding functional regions.

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P43

Machine vision phenotyping of maize seedling growth and morphology

(submitted by Elizabeth Sampson <samps225@umn.edu>)
Full Author List: Sampson, Elizabeth L[1]; Enders, Tara A[2]; Miller, Nathan D[3]; Spalding, Edgar P[3]; Springer, Nathan M[2]; Hirsch, Cory D[1]
[1] Department of Plant Pathology; University of Minnesota; St. Paul, MN, 55108, USA
[2] Department of Plant and Microbial Biology; University of Minnesota; St. Paul, MN, 55108, USA
[3] Department of Botany; University of Wisconsin; Madison, WI, 53706, USA

The ability to link genotype information to phenotypes can be used to improve plant productivity. Current genotyping methods are widespread and efficient, while phenotyping methods have remained mainly laborious, subjective, or expensive. A simple, affordable platform was developed to acquire standardized RGB images of maize seedlings, using minimal equipment and space. Currently, we have developed algorithms to extract numerous biologically relevant traits such as plant height, width, stem diameter, and digital biomass. These digital measurements are well correlated with manual measurements of the same traits. The daily collection of images allows for estimations of growth rate for individual maize seedlings. We applied this system to monitoring growth rate variation among different maize genotypes subjected to temperature stress. The system was also used to measure variation in heterosis for seedling growth traits in a panel of inbred and hybrid genotypes. We established the utility of the system for capturing responses to temperature stress, however other abiotic and biotic stresses are also compatible with the capabilities of this system.

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P44

Machine vision seedling emergence assay for maize seed biology

(submitted by Nathan Miller <ndmiller@wisc.edu>)
Full Author List: Miller, Nathan[1]; Gustin, Jeff[2]; Baier, John[2]; Spalding, Edgar[1]; Settles, Mark[2]
[1] Department of Botany, University of Wisconsin-Madison, Madison, WI
[2] Horticultural Sciences Department, University of Florida, Gainesville, FL

Seedling emergence is a critical stage in the establishment of a successful crop. Germination and robust seedling establishment are traits selected for during the development of new varieties but with inefficient, largely manual methods. We have developed a machine-vision platform that automatically measures the emergence rate, percent total emergence, variance of emergence, and time until maximal emergence. Each seed is isolated into a single cell, which allows the system to monitor emergence of every seed independently. This level of sample tracking can be leveraged in cases where other data is associated with individual kernels such as kernel shape, near-infrared absorbance profile, or genetic information. With the current system, one camera monitors 168 cells simultaneously. This system includes emergence extraction algorithms that reliably and precisely detect the moment of emergence. The assay has been designed and tested with maize, but could be extended to other plant species. The assay is scalable, can accommodate the application of chemical or environmental treatments, and can be used with different soil types. This high-throughput system will enable the discovery of genes controlling early establishment of plants by increasing the rate and precision with which emergence can be monitored.

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P45

MaizeCODE: From linear sequence to active regions of the maize genome

(submitted by Carrie Davis <davisc@cshl.edu>)
Full Author List: Gingeras, Thomas R.[1]; Jackson, David[1]; Martienssen, Robert[1]; McCombie, W. Richard[1]; Schatz, Michael[3]; Ware, Doreen[1]; Micklos, Dave[1]; Birnbaum, Ken[2]; Consortium, MaizeCODE[1]
[1] Cold Spring Harbor Laboratory, 1 Bungtown Rd. Cold Spring Harbor, NY 11724
[2] New York University, 12 Waverly Place Room 606, New York NY 10003
[3] Johns Hopkins University, Baltimore MD, 21211, USA

Maize (corn) is one of the most economically and agriculturally important crops grown in the world. It has assumed this position after centuries of careful genetic breeding to enhance many of its growth and nutritional properties. The generation of high quality genome sequences paired with diverse molecular data allows scientists to better understand the effects of this selective breeding at both the genetic and epigenetic levels.
The MaizeCODE consortium is a collaborative effort between several research and computational groups and funded by the National Science Foundation (NSF). MaizeCODE investigators aim to build a highly refined genome assembly for several maize strains (B73, NC350, W22, and Til 11) and to identify active regions of these genomes using several assays in various tissue and developmental timepoints. MaizeCODE investigators with expertise in whole-genome sequencing, RNA-Seq, Histone Modification ChIP-Seq and Transcription Factor ChIP-Seq and DNA Methylation will collectively profile the respective samples types using Illumina, 10X and PacBio technologies.
The data will serve as a public resource and be made available in an unrestricted fashion through a CyVerse MaizeCODE project portal (in development) for the plant community. The data will be accompanied by extensive structured metadata in order to capture relevant experimental and analysis/file properties in an effort to promote the greatest level of transparency and community uptake and usability. Analysis pipelines and methods are also in development. The infrastructure the MaizeCODE consortia is developing (database, metadata, methods, pipelines, etc…) can serve as a foundation for other large-scale genome projects in maize and other plants.
These efforts will lead to the discovery of novel gene, transcripts, binding sites, and epigenetic marks and will be used by the community to build and improve gene annotations, understand expression profiles in a variety of contexts ultimately leading to improved crops. A project overview will be presented.

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P46

Mining auxin response factor binding sites in maize using informatics approaches

(submitted by Sidharth Sen <ssz74@mail.missouri.edu>)
Full Author List: Sen, Sidharth[1]; Galli, Mary[2]; Gallavotti, Andrea[2 3]; Joshi, Trupti[1 4]
[1] Informatics Institute, University of Missouri Columbia, Columbia, MO, USA, 65211
[2] Waksman Institute of Microbiology, Rutgers University, Piscataway, NJ, USA 08854
[3] Dept. of Plant Biology and Pathology, Rutgers University, New Brunswick, NJ, USA 08901
[4] Department of Molecular Microbiology and Immunology, School of Medicine, University of Missouri Columbia, Columbia, MO, USA 65212

DNA affinity purification sequencing (DAP-seq) is a recently developed technique for transcription factor (TF) binding site discovery that produces ChIP-seq type data. A major advantage of the DAP-seq method is that it uses exogenously expressed TFs to directly interrogate genomic DNA, thereby circumventing the need for tagged transgenic lines or gene-specific antibodies while still capturing TF binding events in their genomic sequence context. This method is being utilized to generate genome wide binding profiles of ten different maize AUXIN RESPONSE FACTORS (ARFs). ARFs are responsible for activating or repressing auxin response genes and play an important role in growth and developmental processes.
We have applied informatics approaches to mine the ARF DAP-seq datasets to better understand how this important family of TFs regulates gene expression. High quality reads after filtration were aligned against maize reference genome (B73 V2 5b). These aligned reads were then used to call peaks and motifs within the peak regions using two popular tools - MACS (V2.1.1) and GEM (V2.7) respectively. Our analysis includes investigation of patterns underlying ARF binding with respect to the presence and position of binding motifs. ARFs are known to bind as dimers to pairs of TGTC motifs as direct repeats, inverted repeats and everted repeats. Based on this knowledge, we analyzed our motif dataset using custom python scripts to find specific genomic regions with such signatures. This has generated a highly constrained list of target genes and their nearest ARF binding sites and forms the basis for future downstream analysis and functional enrichment to understand the specificity of ARF function.

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P47

Mining maize with Gramene

(submitted by Joshua Stein <steinj@cshl.edu>)
Full Author List: Stein, Joshua[1]; Wei, Sharon[1]; Jiao, Yinping[1]; Wang, Bo[1]; Campbell, Michael[1]; Tello-Ruiz, Marcela Karey[1]; Olson, Andrew[1]; Kumari, Sunita[1]; Keays, Maria[2]; Petryszak, Robert[2]; Kersey, Paul K[3]; Jaiswal, Pankaj[3]; Doreen, Ware[1 4]
[1] Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, USA
[2] EMBL-EBI, Hinxton, UK
[3] Oregon State University, Corvallis, OR, USA
[4] USDA ARS NAE Robert W. Holley Center for Agriculture and Health, Ithaca, NY, USA

The Gramene database (http://www.gramene.org) is an integrated resource for comparative genome and functional analysis in plants. The database provides agricultural researchers and plant breeders with valuable biological information on genomes and plant pathways of numerous crops and model species - including maize - thus enabling powerful comparisons across species. In addition to maize B73 (RefGen_V4), the database also includes reference genomes of sorghum, rice, wheat, Brachypodium, Setaria, and dozens of other plant species. New data associated with the RefGen_V4 release includes: i) subgenome designation and ohnologs, ii) full-length transposable element annotations, and iii) gene ID history table (v3 ⇔ v4). Annotation tracks include methylome signatures, genome-wide long non-coding RNAs, and nascent transcriptomes. We also added two pairs of synteny for Z. mays vs Setaria italica and Brachypodium distachyon. Gramene is also a resource for variation data. The current release includes the maize HapMap2 (~55 million SNPs in 104 lines) and Panzea's 2.7 GBS (~720K SNPs in 16,718 lines) variation data sets. In the last year, we added new variation in rice. Gramene has also developed an integrated search database and modern user interface that leverage these diverse annotations to allow scientists to find genes through selecting auto-suggested filters. The interface offers interactive views of the search results both in aggregate and in the context of a gene in the result set. Gramene's pathway portal, Plant Reactome (http://plantreactome.gramene.org/), hosts ~240 metabolic, signaling, regulatory, and genetic reference pathways, -omics data and pathway comparison analysis tools, and orthology-based projections to over 78,000 gene products in 66 species across the plant kingdom. Both pathway and genome browsers, as well as the search result panel, display EBI-ATLAS baseline gene expression. Gramene is supported by an NSF grant IOS-1127112, and partially from USDA-ARS (1907-21000-030-00D).

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P48

Nkd1, Nkd2 and Opaque2 play essential roles in gene regulatory network of maize endosperm development

(submitted by Hao Wu <haowu@iastate.edu>)
Full Author List: Wu, Hao[1]; Adhikari, Bibechana[1]; Zhan, Junpeng[2]; Li, Guosheng[2]; Yadegari, Ramin[2]; Becraft, Philip W.[1 3]
[1] Genetics, Development & Cell Biology Department, Iowa State University, Ames, Iowa 50011
[2] School of Plant Sciences, University of Arizona, Tucson, Arizona 85721
[3] Agronomy Department, Iowa State University, Ames, Iowa 50011

Maize endosperm is one of the products of double fertilization. During development, it accumulates starch and storage proteins important for germination. Prior studies showed that *opaque2 (o2)* mutants and *nkd1, nkd2* double mutants dramatically altered the endosperm cell fate, tissue differentiation, nutrient biosynthesis and storage, and germination rate. As transcription factors, they directly control and indirectly influence a large number of downstream genes that form a gene regulatory network. Recently, studies revealed that NKD1 and NKD2 could directly activate the expression of *o2* , and NKD2 could negatively regulate the expression of *nkd1* gene. To elucidate the relationship between *nkds* and *o2* , triple mutants were developed by crossing *nkd1, nkd2* double mutants with *o2* single mutants. In F2 generation, the kernel phenotypes in kernel size, surface characteristics, endosperm size, vitreousness, hardness and aleurone layers indicate interaction between NKD1, NKD2 and O2. Weighted gene co-expression network analysis (WGCNA) was performed in a combination of normalized maize RNA-seq data sets, from B73 kernel tissue samples (8 DAP), B73/nkd mutant samples (collected from 15DAP aleurone and starchy endosperm, respectively), B73/*o2* mutant samples (15 DAP whole endosperm), and B73/*thk1* mutant samples (20 DAP whole endosperm). The algorithms identified several gene modules (a group of co-expressed genes) correlated well with a specific sample, indicating that tissue types, mutants and development stages may influence a specific group of co-expressed genes. Further, within a specific module, the analysis identified several hub genes, which may play central role in the module. Referring to prior studies, some hub genes are targets of NKDs and O2, indicating that they could influence several modules in different development stages. Therefore the gene network studies associated with NKDs and O2 will provide a deeper insight into maize endosperm development.

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P49

Optimized reduced representation bisulfite sequencing reveals tissue-specific mCHH islands of maize

(submitted by Fei-Man Hsu <fmhsu0114@gmail.com>)
Full Author List: Hsu, Fei-Man[1 2]; Yen, Ming-Ren[2]; Wang, Chi-Ting[2]; Lin, Chien-Yu[2]; Wang, Chung-Ju Rachel[2]; Chen, Pao-Yang[2]
[1] Graduate School of Frontier Sciences, the University of Tokyo, Chiba 277-8561, Japan
[2] Institute of Plant and Microbial Biology, Academia Sinica, Taipei 11529, Taiwan

DNA methylation is known to play important roles in the development and regulation of maize. To profile genome wide DNA methylation in maize with single base resolution, whole genome bisulfite sequencing (WGBS) is infeasible for its high sequencing cost. Reduced representation bisulfite sequencing (RRBS) was originally developed for mammalian genomes, using MspI digestion with size selection of fragments that are CpG-rich. Here, we first adapt RRBS in maize, one of the major crops with a genome size of 2.5Gb, to study the role of DNA methylation in developmental regulations using shoot and tassel primordium tissues. We developed a pipeline for selecting restriction enzymes in silico, and showed in maize genome MseI and CviQI digested fragments are successfully enriched for promoter and genebody, respectively. Overall we found tassel primordium is more methylated than shoot, indicating extensive new methylation accumulated during development. Differentially methylated regions are found to locate in promoters associating with reproduction and development processes, suggesting a possible role of DNA methylation in regulating vegetative to reproductive transition. Although only about 10 % of differentially methylated genes are correlated with transcriptional changes, we found that highly methylated CHH islands (mCHH islands) from 600-700bp upstream of TSS are positively correlated with differential-gene expression between tassel and shoot. Furthermore, we found that regions between TSS and mCHH island reveal high sequence similarity to TFBSs which regulate the flowering process and the timing of transition from vegetative to reproductive phases. By integrating MNase-seq and smRNA-seq data, we found that mCHH islands are accumulated with 24nt-smRNA, and mark the transition of open chromatin to ensure the accessibility of transcription factors in the promoter regions for tissue specific gene regulation, and the silencing of nearby transposons.

P50

Optimizing genotyping-by-sequencing for gene mapping in polyploids

(submitted by Daniel Wickland <wicklan2@illinois.edu>)
Full Author List: Wickland, Daniel[1]; Hudson, Karen[2]; Hudson, Matthew[1]
[1] Department of Crop Sciences, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA
[2] Department of Agronomy, Purdue University, West Lafayette, IN 47907, USA

Genotyping-by-sequencing (GBS), a method to identify genetic variants and quickly genotype samples, reduces genome complexity by using restriction enzymes to subset the genome into fragments whose ends are sequenced on next-generation sequencing platforms. GBS uses a relatively simple protocol for library preparation and reduces costs by multiplexing samples. However, incomplete genomic data and complex bioinformatics analysis have hindered the widespread adoption of GBS in gene mapping studies. Moreover, in polyploids with homeologous regions resulting from genome duplication, GBS SNP-calling tools may align reads to the wrong homeolog or fail to distinguish between-sample SNPs from within-sample SNPs. These "homeoSNPs" generate background noise that encumbers gene mapping. We have addressed these concerns by developing a GBS analysis pipeline that outperforms widely used pipelines in both accuracy and number of SNPs identified. We compared the performance of various pipelines using Illumina sequence data from a biparental F2 population in soybean, a recent polyploid. Our GBS pipeline called the greatest number of accurate SNPs, with SNP calls over 90% concordant with whole-genome sequencing of the parents. Using our pipeline on this F2 population, we have mapped a gene that affects soybean internode length to chromosome 17. We are refining the map position of this gene using molecular markers.

P51

Population genomics of copy number variation in a natural population of teosinte

(submitted by Wenbin Mei <wbmei@ucdavis.edu>)
Full Author List: Mei, Wenbin[1]; Renny-Byfield, Simon[1]; Lorant, Anne[1]; Springer, Nathan M.[2]; Doebley, John[3]; Ross-Ibarra, Jeffrey[1 4]
[1] Department of Plant Science, University of California, Davis, CA, USA 95616
[2] Department of Plant Biology, University of Minnesota, St Paul, MN, USA
[3] Department of Genetics, University of Wisconsin-Madison, WI, USA 53706
[4] Center for Population Biology and the Genome Center, University of California, Davis, CA, USA 95616

Understanding genetic diversity in natural populations has long been a central theme for biologists. However, so far most efforts have focused on single nucleotide polymorphisms (SNPs) to characterize genetic diversity, demography, introgression and gene flow. Yet considerable evidence suggests an important evolutionary role for structural variation in shaping genetic and phenotypic diversity. Here, we use high depth whole genome sequencing data from a single natural population of the maize wild relative teosinte to study population genomics of copy number variants (CNVs). We find ~1/3 of the genome shows copy number variation in a single population and many CNVs show evidence of segregating in the population. Our initial results suggest open chromatin may increase the rate of deletions. We further show that underlying CNV segregation can dramatically skew frequency estimates of SNPs in the same genomic region. While genome-wide CNVs do appear largely neutral in terms of frequency and localization, some CNVs clearly impact fitness. A small number of CNVs appear to be targeted by positive selection. Taken together, our results begin to shed light on the importance of structural variation in the evolution of large plant genomes.

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P52

Precision farming using high-throughput field phenotyping system

(submitted by Solmaz Hajmohammadi <solmaz.hajmohammadi@lemnatec.de>)
Full Author List: hajmohammadi, solmaz[1]
[1] LemnaTec Corporation, 4240 Duncan Ave Saint Louis, MO 63110

Field phenotyping is the most reliable and comprehensive method to advance crop breeding outcomes and assess agronomic traits that contribute to increased yield. However, phenotyping under field environments is a major bottleneck to basic plant research and agricultural product development. The LemnaTec Field Scanalyzer is a high throughput, fully automated system with a high spectral and spatial resolution imaging sensor arm that accurately positions itself in three dimensions. The sensor arm is equipped with a wide range of sensors and cameras that accurately monitor growth rate, morphology and physiology of plants and field plots. The LemnaTec Field Scanalyzer informs technology development for precision agriculture where the demand for fusing machines, sensors, and crop models is increasing. Specifically, sensor fusion using the Field Scanalyzer is being used to collect meaningful, detailed and dependable information to estimate crop performance, assess soil variability, and quantify germplasm by environment by management (GxExM) interactions. Multi-sensor fusion aims to integrate sensor data collected at different temporal, spectral and spatial scales to produce a dataset with higher order information and knowledge content than could be achieved by assessing each sensor independently. This presentation describes how the LemnaTec Field Scanalyzer and multi-sensor fusion are being used to advance precision agriculture.

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P53

Predicting cleavage activity of CRISPR-Cas9 across diverse maize germplasm using reference genome analysis

(submitted by Ian Braun <irbraun@iastate.edu>)
Full Author List: Braun, Ian R.[1]; Sashital, Dipali[2]; Wang, Kan[3]; Wolt, Jeffrey D.[4]; Lawrence-Dill, Carolyn J.[5]
[1] Predictive Plant Phenomics Program, Iowa State University, Ames, 50011, USA
[2] Roy J. Carver Department of Biochemistry, Biophysics and Molecular Biology, Iowa State University, Ames, 50011, USA
[3] Department of Agronomy, Iowa State University, Ames, 50011, USA
[4] Biosafety Institute for Genetically Modified Agricultural Products, Iowa State University, Ames, 50011, USA
[5] Department of Genetics, Development, and Cell Biology, Iowa State University, Ames, 50011, USA

The CRISPR-Cas9 system enables sequence-specific editing at target sites within a genome. However, editing may also occur at other points in the genome (off-target sites) where sequences are similar. In particular, off-target cleavage presents a problem for specific editing in large, polyploid, or redundant eukaryotic genomes such as that of maize. Exacerbating this problem, target sites for CRISPR-Cas9 often are selected within the context of a reference genome, and then applied to different germplasm altogether. In such cases, it would be valuable to estimate the number of expected off-target sites unique to the new line. We compared the maize reference genome B73 with the genomes of four additional sequenced lines (B104, W22, Mo17, and PH207) to estimate this relationship. The predicted ratios between unique off-target sites and the number of off-target sites in the reference genome are as large as 0.15, depending on the specific germplasm analyzed. These findings likely are influenced by various confounding factors, including the relative quality of genomes analyzed. The extent to which predicted off-target rates vary based on genuine genomic differences versus such artifacts is discussed. Implications for experimental design are described.

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P54

Predicting novel gene regulatory interactions by combining different datasets

(submitted by Fabio Gomez-Cano <gomezcano.1@osu.edu>)
Full Author List: Gomez-Cano, Fabio[1 3]; Gray, John[4]; Doseff, Andrea I.[2 3]; Grotewold, Erich[1 3]
[1] Center for Applied Plant Sciences, The Ohio State University, Columbus, Ohio, 43210
[2] Department of Physiology and Cell Biology, Dorothy M. Davis Heart and Lung Research Institute, The Ohio State University, Columbus, Ohio, 43210
[3] Department of Molecular Genetics, The Ohio State University, Columbus, Ohio, 43210
[4] Department of Biological Sciences, University of Toledo, Toledo, Ohio, 43606

Elucidation of gene regulatory networks (GRNs) is one of major areas in plant systems biology, given the intrinsic relationships between phenotypic traits and particular gene expression profiles. These expression profiles are largely defined by regulatory links between sets of transcription factors (TFs) and their genes targets. In maize, a set of 1,100 protein-DNA interactions (PDIs) among 568 TFs and 54 enzyme genes associated to phenolic metabolism were recently identified[1]. In order to determine new PDIs associated with maize phenolic metabolism, we combined co-expression profiles of the 568 TFs with the entire maize genome, using data from the maize gene expression ATLAS project[2], with the known targets identified for the 568 TFs. The correlation between each pair of TF-gene interactions was calculated using the Pearson's correlation coefficient (PCC). The analyses focused on: 1) Identifying new possible targets for the 568 TFs, which could be experimentally validated; 2) identifying TF combinatorial interactions that control common sets of target genes; and 3) identifying possible hierarchical TF arrangements that impact the control of phenolic biosynthesis genes (e.g., feed-forward loops). We identified several putative novel protein-protein interactions and PDI which are currently being experimentally validated in the lab. The results to be presented highlight the importance of high-quality PDIs and expression datasets to guide predictive biology towards the discovery of novel edges in gene regulatory grids. This research was funded by NSF grant IOS-1125620 to J.G, A.I.D. and E.G.

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P55

QTL of 29 novel traits contributing to drought tolerance identified by both of linkage analysis and GWAS in tropical maize

(submitted by Penghao Wu <pw396@cornell.edu>)
Full Author List: Wu, Penghao[1 2]; Buckler, Edward S[1]; Ribaut, Jean Marcel[3]
[1] Institute for Genomic Diversity, Cornell University, Ithaca, New York 14950
[2] College of Agronomy, Xinjiang Agriculture University, Urumqi, China 830052
[3] Generation Challenge Programme, CIMMYT, Delegacion Cuauhtemoc, Mexico 06600

To improve maize productivity and affiliated food security under water deficit, we conducted a comparative QTL analysis for drought tolerance across six segregating populations involving seven tropical maize inbred lines by linkage analysis and GWAS. There were 29 new traits generated by combining traditional traits evaluation under 43 water-stressed (WS) and 17 well-watered (WW) regimes. Firstly, these new traits were all generally summarized as ratio of classical traits evaluated under WS field divided by the corresponding one under WW condition. Secondly, a total of 182 QTL were detected in single-trial analyses through linkage analysis, 33 of them were plant architecture trait, 69 of them were yield-related trait, 34 of them were flowering time trait, and 45 were physiological-related trait. In the same time, the genotypic data of these 1498 lines within the six population was imputed according to the GBS data of their seven parents. Then combining with the 29 new traits analyzed in linkage analysis, we also conducted genome association analysis in the joint population and detected a total of 2871 associated SNPs, 280 of them were plant architecture trait, 1539 of them were yield-related trait, 420 of them were flowering time trait, and 632 were physiological-related trait. Finally, under the comparison investigation between two methods, there were 1663 SNPs could fit into 24 QTL intervals with corresponding trait. Among these regions, five of them coincide shared contribution to more than two different kinds of traits. In this way, we believed that there should be candidate genes in these regions having the key function in drought tolerance mechanism regulatory.

P56

RefSeq curation at NCBI - adding value to the maize genome resources

(submitted by Brian Smith-White <smtwhite@ncbi.nlm.nih.gov>)
Full Author List: Smith-White, Brian[1]; Pruitt, Kim D.[1]; Murphy, Terence D.[1]; Thibaud-Nissen, Francoise[1]
[1] National Center for Biotechnology Information, National Library of Medicine, National Institutes of Health ; Bethesda, MD 20894

NCBI created Zea mays RefSeq transcript-protein pairs and the associated NCBI Gene record from the INSDC maize accessions. This is a publicly available resource consisting of a non-redundant collection of sequence records for mRNA and the encoded protein; each pair with an associated NCBI Gene record. Curation of Zea mays RefSeq transcripts creates a more robust and accurate collection of genome resources for the maize research community. Curation involves 1) identifying instances of multiple identical RefSeq transcripts for a particular gene and resolving by merging, 2) identifying instances of genes expressing transcript isoforms and creating the necessary RefSeq accessions, 3) identifying instances of RefSeq transcripts with sequence variations relative to the genome sequence and resolving by creating either a new version of the transcript accession or a new transcript accession, 4) identifying instances of structural and/or sequence deficiencies in the genome, 5) identifying instances where the annotation endeavors by NCBI and maizesequence.org differentially annotate a gene, 6) identifying instances of the genome sequence lacking a particular gene and 7) identifying instances where the RefSeq was created from a chimera INSDC accession. Points four and six result in a record in the internal Genome Problem database at NCBI; the contents of which are shared with MaizeGDB. A consequence of points five and six is that NCBI may be able to still track the missing locus in the NCBI Gene resource and be able to provide a representative RefSeq transcript and protein record (i.e. one of the strong advantages to maintaining the transcript-based NM/NP dataset).

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P57

Resources for maize genome annotation: Lessons learned from B73

(submitted by Michael Campbell <mcampbel@cshl.edu>)
Full Author List: Campbell, Michael S[1]; Jiao, Yinping[1]; Wang, Bo[1]; Wei, Xuehong[1]; Chougule, Kapeel[1]; Stein, Joshua C[1]; Ware, Doreen[1]
[1] Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 11724

Genome projects began as large international efforts focused on generating reference genomes for organisms important to humans, including agriculturally important plants. As sequencing costs decreased, efforts have turned to population level re-sequencing to explore genomic diversity. Though informative and adequate for most genetic applications, short read re-sequencing is limited in its ability to identify large structural variation, a hallmark of maize diversity. To explore this important area of maize diversity and evolution, multiple maize lines are being sequenced and assembled de novo. Annotating these genomes is the next step. For the annotation of protein coding genes in the new B73 genome assembly we used the MAKER-P annotation pipeline with transcript data from Sanger, Illumina, and PacBio platforms, protein homology from multiple monocot genomes, and Arabidopsis, and a maize specific repeat library. Comparative phylogenomic analysis reveals that maize contains a deficit of genes not explainable by missing annotation, including many defense-related genes. MAKER-P is available through Cyverse (Discovery Environment and Jetstream), and through XSEDE at TACC (Stampede cluster). We have generated a Jetstream image with MAKER-P installed and all of above evidence preloaded. The B73 annotation is complete and has been accepted by GenBank. Though improved over the version 3 annotations, some inaccuracies remain, such as merged genes--including a classical maize gene, and a hard to annotate single-exon zinc-finger transcription factor. Accurate annotation of alternative transcripts also remains challenging. The current version of the annotations errs on the side of sensitivity where alternate transcription start sites were allowed. Transcripts not expected to generate functional protein products due to alternative splicing events such as intron retention are also included as well as those containing non-canonical splicing events. And finally, tissue specific transcripts are also included. These annotations will be available through GenBank's GRC tools allowing community curation/correction.

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P58

RNA-seq of maize and the fungal pathogen *Setosphaeria turcica*

(submitted by Tyr Wiesner-Hanks <tw372@cornell.edu>)
Full Author List: Wiesner-Hanks, Tyr[1]; Saha, Surya[2]; Wu, Dongliang[1]; Mideros, Santiago[3]; Condon, Bradford[4]; Turgeon, B. Gillian[1]; Nelson, Rebecca[1]
[1] School of Integrative Plant Science, Cornell University, Ithaca, NY 14853
[2] Boyce Thompson Institute, Cornell University, Ithaca, NY 14853
[3] Department of Crop Sciences, University of Illinois at Urbana-Champaign, Urbana, IL 61801
[4] Department of Plant Pathology, University of Kentucky, Lexington, KY 40506

Northern leaf blight (NLB) is a foliar disease of maize, caused by the fungal pathogen *Setosphaeria turcica*. NLB is currently estimated to be the most damaging maize disease in the US, causing an estimated $1.9 billion in economic loss in 2015. To explore the mechanisms of both infection and response, we analyzed the transcriptomes of five combinations of maize lines and *S. turcica* isolates over four time points. These time points span the pathogen's transition from a biotroph slowly growing through the epidermis and mesophyll into a necrotroph that rapidly spreads through the vascular system. This transition is marked by enormous shifts in both host and pathogen transcriptomes, including a shift towards stress mitigation as the pathogen plugs the maize vasculature and rapidly colonizes new tissue. The combinations of host and pathogen revealed both common and unique defense mechanisms towards the two differentially virulent fungal isolates, as well as the enormous role of the *Ht2* resistance gene in suppressing the pathogen. Ongoing work seeks to combine this information with previous mapping studies to identify a candidate *Ht2* gene.

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P59

Role of translational dynamics during photosynthetic differentiation of maize leaf

(submitted by Indrajit Kumar <ikumar@danforthcenter.org>)
Full Author List: Kumar, Indrajit[1]; Chotewutmontri, Prakitchai[2]; Mayfield-Jones, Dustin[1]; Stiffler, Nicholas[2]; Barkan, Alice[2]; Brutnell, Tom[1]
[1] Donald Danforth Plant Science Center, St Louis, MO, USA 63132
[2] Institute of Molecular Biology, University of Oregon, Eugene, OR, USA

The maize leaf develops from the tip to the base and a comparative leaf gradient study provides an opportunity to monitor the process of photosynthetic differentiation. We utilized RNAseq and the recently developed Ribo-Profiling technique to understand the relationship between transcriptional and translational dynamics of nuclear genes along 4 predefined sections of maize leaf (Base, sink-source transition, maturing and Tip). Our data suggests that the Base region is very different both transcriptionally and translationally compared to transition, maturing and Tip. More than 1 K genes showed > 2 fold change in translational efficiency (TE) across these 4 sections. Gene categories under translational regulation included chromatin organization/remodeling, chloroplast, carbohydrate metabolism and photosynthesis. We also observed > 150 genes that showed consistently higher TE and ~ 600 genes that showed consistently lower TE across the leaf gradient. We are currently examining the correlations of TE with miRNA target sites and motifs present in the 5' and 3' UTRs of transcripts to identify potential mechanisms of translational control.

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P60

Searching for parallel signatures of selection during domestication in maize and sorghum

(submitted by Xianjun Lai <xlai3@unl.edu>)
Full Author List: Lai, Xianjun[1 2]; Yan, Lang[1]; Lu, Yanli[2]; Schnable, James C.[1]
[1] Department of Agronomy and Horticulture, University of Nebraska-Lincoln, Lincoln, NE, USA 68583
[2] Maize Research Institute, Sichuan Agricultural University, Chengdu, China 611130

Artificial selection to create crops from wild grass species have targeted many of the same phenotypes during the domestication of different crop species. For a few key single gene phenotypic changes, it has already been possible to test whether the same gene was the target of domestication efforts in related crops. Genes which have been targets of selection during domestication or crop improvement in one species, but not another, may be useful targets for future breeding efforts. Maize and sorghum are two closely related crop species which diverged from a common ancestor approximately 12 million years ago. Almost all maize genes cloned through forward genetics are conserved at syntenic orthologous locations in the genome of sorghum. In each species, previous resequencing has generated large sets of polymorphisms for wild relatives, landraces, and improved lines. Using syntenic orthologs identified between these two species, we have identified genes that show parallel or lineage specific patterns of selection during domestication and crop improvement in each species. The resolution of current population genetic tools is sufficiently high that it may also be possible to observe whether selection on the same gene in both lineages are consistently targeted in the same way (ie both changes in protein coding regions or both changes in associated noncoding sequence) or whether the function of same genes were changed through alteration of protein coding exons in one species and noncoding regulatory regions in another.

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P61

Sequencing, assembly, and annotation of B104, a maize transformation resource

(submitted by Nancy Manchanda <nancym@iastate.edu>)
Full Author List: Manchanda, Nancy[1]; Andorf, Carson M[2]; Woodhouse, Margaret[2]; Ye, Liang[3]; Rounsley, Steve S[3]; Wang, Kan[1]; Lawrence-Dill, Carolyn J[1]
[1] Iowa State University, Ames, IA 50011, USA
[2] USDA-ARS, Iowa State Univeristy, Ames, IA 50011, USA
[3] Dow AgroSciences, Indianapolis, IN 46268, USA

Here we report a draft B104 genome sequence, construction of B104 pseudomolecules based on utilization of syntenic relationship between B104 and B73, and our efforts towards comparison of B104 and B73 genome assemblies and annotations. Why B104? It is well known that maize transformation is not straightforward. Many lines can not be cultured and transformed readily, which limits the germplasm available for genome engineering. Adding to the difficulty, the B73 maize genome reference sequence is not readily transformed. B104 is a transformable maize line derived from the same populations as B73. In recent years, the Iowa State University Plant Transformation Facility (http://agron-www.agron.iastate.edu/ptf/) has offered B104 transformation as a first step toward bringing genomics resources to bear on maize transformation. In the interest of the maize research community, a beta-versioned draft assembly and associated B104-based genomics resources are now publicly available at MaizeGDB (http://www.maizegdb.org/gbrowse/maize_b104_chr) under the Toronto Agreement to enable pre-publication data sharing. PacBio single molecule sequencing is currently underway and a full assembly and version 1 release is anticipated in the near future along with an accompanying published description that would allow others to share their genome-scale analyses involving the B104 inbred line.

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P62

Statistical approaches to identifying differentially regulated orthologs (DROs) across related grass species

(submitted by Yang Zhang <yzhang91@unl.edu>)
Full Author List: Zhang, Yang[1]; Qiu, Yumou[2]; Schnable, James[1]
[1] Center for Plant Science Innovation and Department of Agronomy and Horticulture, University of Nebraska, Lincoln, Nebraska, USA, 68588
[2] Department of Statistics, University of Nebraska, Lincoln, Nebraska, USA, 68588

RNA-seq based analyses of gene expression were first widely used to compare patterns of gene express between either two different environments, two different tissue types, or two different genotypes. Many statistical approaches were developed to identify differentially expressed genes (DEGs) between these types of paired data. Comparisons of gene regulation across species are sometimes conducted by comparing sets of DEGs identified in different experiments. Here we show that this type of analysis identifies many genes which do not, in fact, show significant differences in regulatory pattern between experiments. Instead, by testing for the statistical significance of the difference in pattern of expression between orthologous genes in different species subjected to the same stresses, it is possible to identify differentially regulated orthologs (DROs). These can include cases where analysis at the single species level classified neither, one, or both orthologs as a DEG. Applying DRO-based analysis to a dataset of cold stress responsive gene expression in maize (*Zea mays*), sorghum (*Sorghum bicolor*), and foxtail millet (*Setaria italica*), we show that most conserved genes that respond transcriptionally to cold stress do so only in a single lineage, suggesting that many transcriptional responses to cold may be selectively neutral. The DRO concept can also be applied to comparisons of allelic variation across different genotypes in the same species. Using paired data from Mo17 and B73, a DRO based analysis shows that only 39% of genes identified as differentially expressed in only one accession showed significant differences in regulation between accessions, while 23% of genes classified as differentially expressed in both accessions still showed significant differences in regulation between accessions.

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P63

The complex sequence landscape of maize revealed by single molecule technologies

(submitted by Yinping Jiao <yjiao@cshl.edu>)
Full Author List: Jiao, Yinping[1]; Peluso, Paul[2]; Shi, Jinghua[3]; Liang, Tiffany[3]; Stitzer, Michelle C.[4]; Wang, Bo[1]; Campbell, Michael S.[1]; Stein, Joshua C.[1]; Wei, Xuehong[1]; Chin, Chen-Shan[2]; Guill, Katherine[5]; Regulski, Michael[1]; Kumari, Sunita[1]; Olson, Andrew[1]; Gent, Jonathan[6]; Schneider, Kevin L.[7]; Wolfgruber, Thomas K.[7]; May, Michael R.[8]; Springer, Nathan M.[9]; Antoniou, Eric[1]; McCombie, Richard[1]; Presting, Gernot G.[7]; McMullen, Michael[5]; Ross-Ibarra, Jeffrey[10]; Dawe, R. Kelly[6]; Hastie, Alex[3]; Rank, David R.[2]; Ware, Doreen[1 11]
[1] Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724
[2] Pacific Biosciences, Menlo Park, CA 94025
[3] BioNano Genomics, San Diego, CA 92121
[4] Department of Plant Sciences and Center for Population Biology, University of California, Davis, Davis, CA 95616
[5] USDA-ARS, Plant Genetics Research Unit, Columbia, MO 65211
[6] University of Georgia, Athens, Georgia 30602
[7] Department of Molecular Biosciences and Bioengineering, University of Hawaii, Honolulu, HI 96822
[8] Department of Evolution and Ecology, University of California, Davis, CA 95616
[9] Department of Plant Biology, University of Minnesota, St. Paul, MN 55108
[10] Department of Plant Sciences, Center for Population Biology, and Genome Center, University of California, Davis, CA 95616
[11] USDA-ARS, NEA Robert W. Holley Center for Agriculture and Health, Cornell University, Ithaca, New York 14853

Complete and accurate reference genomes and annotations provide fundamental tools for characterization of genetic and functional variation. These resources facilitate elucidation of biological processes and support translation of research findings into improved and sustainable agricultural technologies. Many reference genomes for crop plants have been generated over the past decade, but these genomes are often fragmented and missing complex repeat regions. Here, we report the assembly and annotation of maize, a genetic and agricultural model species, using Single Molecule Real-Time (SMRT) sequencing and high-resolution optical mapping. Relative to the previous reference genome, our assembly features a 52-fold increase in contig length and significant improvements in the assembly of intergenic spaces and centromeres. Characterization of the repetitive portion of the genome revealed over 130,000 intact transposable elements (TEs), allowing us to identify TE lineage expansions unique to maize. Gene annotations were updated using 111,000 full-length transcripts obtained by SMRT sequencing. In addition, comparative optical mapping of two other inbreds revealed a prevalence of deletions in the low gene density region and maize lineage-specific genes.

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P64

The history of maize domestication and adaptation as revealed by a genome-wide survey of SNP variation

(submitted by Gen Xu <jsycxugen@163.com>)
Full Author List: Xu, Gen[1]; Li, Huihui[2]; Li, Lin[3]; Warburton, Marilyn L.[4]; Taba, Suketoshi[5]; Wen, Weiwei[3]; Li, Jiansheng[1]; Yan, Jianbing[3]; Yang, Xiaohong[1]
[1] National Maize Improvement Center of China, China Agricultural University, Beijing 100193, China
[2] Institute of Crop Science, Chinese Academy of Agricultural Sciences, Beijing 100081, China
[3] National Key Laboratory of Crop Genetic Improvement, Huazhong Agricultural University, Wuhan 430070, China
[4] USDA ARS Corn Host Plant Resistance Research Unit, Box 9555, Mississippi State, MS, 39762, USA
[5] International Maize and Wheat Improvement Center (CIMMYT), Apdo. Postal 6-641, Mexico, D.F., 06600, Mexico

Modern cultivated maize (*Zea mays* L. ssp. *mays*) has been heavily selected during domestication and adaptation. To better understand these processes, we conducted a genome-wide survey of 982 maize inbred lines and 190 teosinte accessions using over 40,000 SNP markers. Population structure, principal component analysis, and phylogenetic trees all consistently reflected historical evolutionary relationships among *Zea* species and subspecies. Shared haplotype analysis showed similar high levels of gene flow from Z. *mays* ssp. *parviglumis* and ssp. *mexicana*, confirming the critical contribution of ssp. *mexicana* to the maize gene pool. Scans for selection signatures identified 319 domestication sweeps and 406 adaption sweeps by analysis of wild, tropical and temperate maize, as well as with a set of some known genes such as *tb1*, *pbf1*. To verify the phenotypic effect of the selected regions, we compared the previous reported flowering time QTLs with selective sweeps, 196 domestication-selective sweeps and 238 adaption-selective sweeps were located within known flowering time QTL regions. Furthermore, a genome-wide association study on flowering time related traits were performed, 6 significant association signals were detected within the identified selective sweeps. Inverted chromosome segments provide an opportunity to look for evidence of natural selection, eight long-range inversions were detected based on unusual patterns of linkage disequilibrium (LD) in the wild Z. *mays* subspecies *parviglumis* and *mexicana*. All these results will provide insights into the evolutionary history of maize and be valuable for future maize breeding programs.

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P65

The inverse effect across phyla

(submitted by Adam Johnson <afj8c8@mail.missouri.edu>)
Full Author List: Islam, Soliman[1]; Hou, Jie[1]; Johnson, Adam F[2]; Kanno, Tatsuo[3]; Chen, Pao-Yang[3]; Matzke, Antonius[3]; Matzke, Marjori[3]; Cheng, Jianlin[1]; Birchler, James A[2]
[1] Department of Computer Science, University of Missouri, Columbia, MO 65211
[2] Division of Biological Sciences, University of Missouri, Columbia, MO 65211
[3] Institute of Plant and Microbial Biology, Academia Sinica, Taipei, Taiwan

The inverse dosage effect was described in maize decades ago. A dosage series of the long arm of chromosome 1L produced an inverse correlation of selected gene expression located in trans across the genome. Experiments in Drosophila showed similar results and single genes, primarily transcription factors, signal transduction components and chromatin proteins, were identified that produced this effect on target genes. RNA-Seq experiments in maize and Drosophila indicate that global trans effects of trisomy can be varied but the most common deviation from normal is a reduced expression more or less within the inverse range (down to 0.67) although outlying peaks are also found that extend positively and negatively. The Matzke lab conducted RNA-Seq on the five trisomies of Arabidopsis. When ratio distributions are calculated, the inverse effect in trans is evident in each trisomy, most prominently with trisomies 1 and 4 and least in trisomy 5. These experiments were conducted such that, to the extent possible, absolute amounts of gene expression were sought to produce the ratio distributions given the knowledge that trans-acting reductions are common. Studies in other laboratories had determined gene expression in disomic haploids in yeast and trisomics in mice but the data analysis normalized the varied cis gene expression by that from the unvaried genes in trans assuming that no modulations across the genome occur. This normalization technique would minimize the ability to recognize the inverse effect because it is canceled. Thus, we re-analyzed the absolute levels from these yeast and mouse studies to produce ratio distributions. Again, there is a wide spectrum of gene modulations in trans that are observed but the most common effect is a reduced gene expression in trans by hyperploidy. The recognition of similar aneuploidy effects in monocots, dicots, fungi, insects and mammals suggests that these effects are a general reflection of an imbalance of regulatory gene products in the processes of gene expression. Research supported by NSF grant IOS-1545780.

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P66

The maize Genomes to Fields (G2F) Initiative: Data management and availability

(submitted by Carolyn Lawrence-Dill <triffid@iastate.edu>)
Full Author List: Alkhalifah, Naser[1 8]; Campbell, Darwin A.[1 8]; Gardiner, Jack[2 8]; Romay, Cinta[3 8]; Rothfusz, Emily[4 8 9]; Walls, Ramona[5 8]; Walton, Renee[1 8]; Yeh, Ching-Ting[1 8]; Edwards, Jode[6]; Ertl, David[7]; Schnable, Patrick S.[1]; de Leon, Natalia[4]; Lawrence-Dill, Carolyn J.[1]
[1] Iowa State University
[2] MaizeGDB & University of Missouri
[3] Cornell University
[4] University of Wisconsin
[5] CyVerse & University of Arizona
[6] USDA/ARS
[7] Iowa Corn Promotion Board
[8] these authors listed alphabetically
[9] project coordinator

The multi-institutional Genotype by Environment (GXE) subproject of the maize Genomes to Fields (G2F) Initiative aims to assess the impacts of genotype and environmental effects on the performance of a large collection of maize hybrids. Toward that aim, we have collected and analyzed genotypic, phenotypic, and environmental data from more than 30 North American field locations across 3 years (a total of 86 environments). These data comprise 14 core phenotypic traits and weather measurements combined with genotypic data, and for a subset of locations, image data (at scales from individual plant to individual field). To assist in the management of these diverse data types, we have developed and deployed a robust yet flexible data management and analysis pipeline that meets the project's needs but is also extensible to the broad plant breeding community. In this poster, we present progress made over the past year working with partners at CyVerse and describe methods for G2F data access and analysis. For more information, visit http://www.genomes2fields.org/.

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P67

The Maize TFome - development of a transcription factor open reading frame collection for functional genomics

(submitted by John Gray <jgray5@utnet.utoledo.edu>)
Full Author List: Gray, John[4]; Goetting-Minesky, Mary P.[4]; Li, Tai[4]; Velliquette, David[4]; Thomas, Julie[4]; Burdo, Brett[1]; Wittler, Bettina[1]; Hunt, Matthew[1]; Gentzel, Irene[1]; dos Santos Brito, Michael[1]; Mejía-Guerra, Maria K.[1]; Connolly, Layne N[1]; Qaisi, Dalya[1]; Casas, Maria I.[2]; Li, Wei[1]; Doseff, Andrea I.[2 3]; Grotewold, Erich[1 2]
[1] Center for Applied Plant Sciences (CAPS), The Ohio State University, Columbus, OH 43210
[2] Department of Molecular Genetics, The Ohio State University, Columbus, OH 43210
[3] Department of Physiology and Cell Biology, Heart and Lung Research Institute, The Ohio State University, Columbus, OH 43210
[4] Department of Biological Sciences, University of Toledo, Toledo, Ohio 43606 USA

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, and nutritional health. Transcription Factors (TF) and co-regulators (CoReg) represent ~7% of the maize genome (~3000 genes) are key regulators of plant metabolic function. To define the gene regulatory networks (GRNs) that regulate metabolism of maize phenolic compounds, we initiated The Grass Transcription Factor ORFeome Project (TFome). We report the development and release of a publicly available maize TF ORF collection (TFome) of 2,034 clones corresponding to 2,017 unique gene models in recombination-ready vectors that make possible the facile mobilization of the TF sequences into a number of different expression vectors. The collection also includes several hundred co-regulators (CoREG) for which we propose a standard nomenclature, as we have previously done for TFs. Strategies were developed to overcome the limitations associated with cloning ORFs from a genome that remains incompletely annotated, with a partial full-length cDNA set available, and with many TF/CoREG genes lacking experimental support. This required, in many instances, combining genome-wide expression data with gene synthesis approaches. The strategies developed will be valuable for developing similar resources for other agriculturally important plants. Information on all the clones generated is available through the GRASSIUS knowledgebase (http://grassius.org/). To date the entire collection has been requested 7 times and a total of ~15,000 clones have been 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 (http://dx.doi.org/10.1016/j.molp.2016.10.020). The constructions and release of the Maize TFome has been described in Burdo et al., The Plant Journal. 2014 80(2):356-66 and Gray et al., Bio-Protocol 2015 Vol 5, Issue 15. This project was funded by NSF grant IOS-1125620.

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P68

The role of differential fractionation in maize genome content variation

(submitted by Alex Brohammer <broha006@umn.edu>)
Full Author List: Brohammer, Alex B.[1]; Kono, Thomas J.Y.[1]; McGaugh, Suzanne E.[2]; Springer, Nathan M.[3]; Hirsch, Candice N.[1]
[1] Department of Agronomy and Plant Genetics, University of Minnesota, St. Paul, MN 55108
[2] Department of Ecology, Evolution, and Behavior, University of Minnesota, St. Paul, MN 55108
[3] Department of Plant and Microbial Biology, University of Minnesota, St. Paul, MN 55108

Maize is an ancient allotetraploid produced through a hybridization event between two progenitor species. Fractionation through extensive gene loss following whole-genome duplication has led to many of the maize-sorghum syntenic orthologs being present in only one of the two underlying maize subgenomes. The differential fractionation of homeologous genes among maize inbreds is one mechanism through which the considerable genome content variation observed in maize may have arisen. In this study, we show that two elite inbred lines, B73 and PH207, share extensive similarities in syntenic block composition compared to sorghum. A detailed analysis of synteny with sorghum revealed 1,489 genes are differentially fractionated between these two genomes. Many of the genes involved in differential fractionation exhibit expression consistent with other syntenic genes not involved in differential fractionation. We also found evidence for extensive genome content variation between these inbred lines for non-syntenic genes. Expression analysis of non-syntenic genes confirmed previous observations that non-syntenic genes are on average expressed lower, more variable, and expressed in fewer tissues than syntenic genes. Thus, differential fractionation is only one of the mechanisms underlying the high levels of genome content variation observed in maize. However, given evidence that supports that variation in syntenic genes contributes to phenotypic variation, these genes likely represent an important class of genome content variants for future functional studies.

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P69

The unique transcriptome and genome structure of maize *Ufo1* mutant

(submitted by Kameron Wittmeyer <ktw5072@psu.edu>)
Full Author List: Wittmeyer, Kameron[1]; Tan, Qixian[1]; Cui, Jin[1]; Xue, Weiya[1]; Jiao, Yinping[2]; Lee, Tzuu-fen[3 5]; Meyers, Blake[3 4]; Ware, Doreen[2 6]; Chopra, Surinder[1]
[1] Department of Plant Science, Pennsylvania State University, University Park, PA 16802
[2] Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724
[3] The Donald Danforth Plant Science Center, St. Louis, MO 63132
[4] Division of Plant Sciences, University of Missouri, Columbia, MO 65211
[5] DuPont Pioneer, Des Moines, IA 50131
[6] USDA-ARS, PSNR, Ithaca, NY 14853

The maize mutant *Unstable factor for orange1* (*Ufo1*) is a modifier of tissue specific gene expression. Presence of *Ufo1* can be monitored by overexpression of *pericarp color1* (*p1*) as a reporter. The common feature of *Ufo1-p1* interactions is that epigenetically silent *p1* alleles *p1-wr, p1-rr', p1-pr, p1-ww*,* and *p1-pr^{jp}* are up regulated. In the case of *p1-wr*, *Ufo1* can induce ectopic expression in tissues where *p1* does not normally express. *P1-wr;Ufo1* plants show several pleiotropic developmental defects. To further understand the role of *Ufo1* in plant growth and development global gene expression studies were performed on three tissues: seedlings, leaves, and pericarps. These transcriptomes were also compared with those of *P1-rr* to determine how much of the *Ufo1*-effect is due to overexpression of *p1*. More than 11,000 genes were found to be differentially expressed (FDR <.1) in *P1-wr;Ufo1*. Overall transcriptome profiles of *P1-wr;Ufo1* across different tissues is very similar to *P1-rr,* affecting three major GO categories by up regulating phenylpropanoid and flavonoid biosynthesis and by down regulating ribosomal proteins and DNA replication. Interestingly, *Ufo1* up-regulates genes enriched for GO categories for response to various stimuli such as ROS and high light intensity which are not found in transcriptomes of *P1-rr*. The GO enrichment profiles suggest plants under chronic stress, which may explain the pleiotropic defects found in *Ufo1* plants. Ongoing efforts to map and clone *Ufo1* using high density SNP data has revealed a ~30 Mb region of identity by decent (IBD) with 14 maize inbred lines that contains the centromere on chromosome 10 and most of the mapping region of *Ufo1*. A combination of RNA-seq data and genomic PacBio sequence data are being used to identify *ufo1* candidate genes.

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P70

Transformation and multimerization of large linear molecules via bombardment

(submitted by Natalie Nannas <njnannas@uga.edu>)
Full Author List: Nannas, Natalie J.[1]; Liu, Jianing[1]; Aspinwall, Brooke[1]; Dawe, R. Kelly[1]
[1] University of Georgia, 120 East Green St. Athens, GA 30602

Transformation is a critical technique to insert new genetic material or modify the existing genome. Plants are typically transformed via *Agrobacterium* or particle bombardment. Due to the greater stability of transgene insertion and expression by *Agrobacterium*, fewer investments have been made in understanding and improving biolistic transformation. Yet, biolistic transformation offers distinct advantages: it can be used on a broader range of plants species and strains and DNA can be transformed in a variety of sizes, forms and combinations. To date, no study has fully determined the integrity and insertion status of DNA introduced via bombardment. Using whole genome sequencing, we characterized the insertion of the 48kb linear lambda phage genome into *Zea mays* and *Orzya sativa*. In addition to determining the number and distribution of insertions and the degree of degradation and breakage, we also tested for multimerization of the lambda molecule. The ends of the lambda genome have *cos* sites, or single stranded "sticky ends", that allow the molecule to circularize or multimerize. We were able use these sticky ends to insert multimerized lambda molecules into the plant genome; this type of molecule multimerization could help stitch together large transgenic constructs for transformation.

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P71

Unbiased K-mer analysis reveals changes in copy number of highly repetitive sequences during maize domestication and improvement

(submitted by Sanzhen Liu <liu3zhen@ksu.edu>)
Full Author List: Liu, Sanzhen[1]; Zheng, Jun[2]; Migeon, Pierre[1]; Ren, Jie[1]; Hu, Ying[1]; He, Cheng[2]; Liu, Hongjun[3 4]; Fu, Junjie[2]; White, Frank F[5]; Toomajian, Christopher[1]; Wang, Guoying[2]
[1] Department of Plant Pathology, Kansas State University, Manhattan, KS. 66506. U.S.A.
[2] Institute of Crop Science, Chinese Academy of Agricultural Sciences, Beijing 100081, P.R.China
[3] State Key Laboratory of Crop Biology, Shandong Key Laboratory of Crop Biology, Taian 271018, P.R. China
[4] College of Life Sciences, Shandong Agricultural University, Taian 271018, P.R. China
[5] Department of Plant Pathology, University of Florida, Gainesville, FL. 32611. U.S.A.

The most complex components of genomes are repetitive elements, which are largely recalcitrant to characterization. A k-mer approach, which is independent of a reference genome, was devised and used to compare highly repetitive sequences among maize lines. Raw whole genome shotgun (WGS) sequences of B73 and Mo17 were first analyzed as a proof of principle. Significant differences were identified in highly repetitive sequences, including centromere, 45S ribosomal DNA (rDNA), knob, and telomere repeats, in addition to substantial differences in low copy sequences. Novel genotype specific 45S rDNA k-mers were also discovered. B73 and Mo17 specific k-mers were used to examine allele-specific expression of 45S rDNA in B73xMo17 hybrids. Although Mo17 contains a higher copy number than B73, accumulation levels of overall 45S rDNA are similar, indicating that transcriptional or post-transcriptional regulation mechanisms likely compensate for copy number differences in the hybrids. Using WGS sequences of B73xMo17 doubled haploids, genomic locations for differential repetitive loci were genetically mapped, revealing different organization of highly repetitive sequences in the two genomes. A k-mer analysis of WGS sequences of HapMap2 lines, including maize wild progenitor, landraces, and improved lines, was then performed, revealing decreases and increases in repetitive elements associated with the centromere, 45S rDNA, knob loci, and retrotransposons. The results indicate extensive changes in diverse genomic repeats during maize domestication and improvement. Our results also provide an unbiased and novel genome comparative method to mine massive sequencing data for biological insights.

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P72

Uncovering new players in the auxin pathway through WGCNA analysis

(submitted by Katherine Guthrie <klgdn2@mail.missouri.edu>)
Full Author List: Guthrie, Katherine[1]; Sen, Sidharth[2]; Yao, Hong[1]; Wang, Qingyu[3]; Altman, Naomi[3]; Joshi, Trupti[1 2]; McSteen, Paula[1]
[1] Bond Life Sciences Center, Department of Biological Sciences, University of Missouri – Columbia, Columbia, MO 65211
[2] Missouri Informatics Institute, University of Missouri – Columbia, Columbia, MO, 65211
[3] Department of Statistics, Penn State University, University Park, PA 16802

Plant development occurs continuously due to the action of meristems, groups of stem cells that are constantly dividing and differentiating. The shoot apical meristem (SAM) is responsible for vegetative growth, and upon the switch to reproductive growth, the SAM transitions into the inflorescence meristem (IM), which gives rise to the tassel (male inflorescence). The development of organs by meristems requires the phytohormone, auxin. While research has been done to elucidate the components of the auxin signaling pathway in maize, there is still a lot to uncover, specifically the target genes controlling organogenesis. We aim to elucidate new components of the auxin pathway by identifying changes in gene regulation when a known step of the pathway is affected. To this end, we analyzed the transcriptomes of the top and bottom 1 mm of 3-4 mm tassels of the auxin mutants *barren inflorescence2* (*bif2*), *barren stalk1* (*ba1*) and *barren stalk2* (*ba2*). *ba1* and *ba2* interact with each other and function downstream of auxin, while *bif2* functions in auxin transport and regulation of *ba1*. We identified differentially expressed (DE) genes compared to B73, and performed Weighted Gene Co-Expression Network Analysis (WGCNA), to identify clusters of co-expressed genes. Gene Ontology analysis on these clusters identified significantly enriched terms, such as transcriptional regulation, indicating biological relevance. Finally, the co-expression network was visualized in Cytoscape. Together these analyses provide a better understanding of the auxin regulatory network in maize.

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P73

Understanding variance of maize traits at multiple biological scales

(submitted by Elena Rice <elena.a.rice@monsanto.com>)
Full Author List: Rice, Elena[1]; Tucker, Sarah[1]; Dohleman, Frank[1]; Grapov, Dmitry[1]; Flagel, Lex[1]; Yang, Sean[1]; Wegener, Kimberly[1]; Kosola, Kevin[1]; Swarup, Shilpa[1]; Hall, Mike[1]; Allen, Edwards[1]
[1] Monsanto Company, St. Louis, MO, 63017

There are increasing opportunities to improve crop yield using diverse approaches from traditional plant breeding to recent technological developments such as genome editing, microbial enhancement, as well as traditional biotechnology. Utilizing these technologies requires understanding of traits that can contribute to an integrative phenotype like yield under diverse environmental conditions. The key challenge here is the ability to measure large number of physiological and molecular traits using sufficiently diverse and commercially-relevant populations, and the ability to integrate genotype, phenotype, and environmental variables. Here, we systematically collected, 31 phenotypic traits including yield, 83 annotated metabolites, over 21,000 transcripts and 529 genera of microbes on a set of 57 diverse, commercially relevant maize hybrids across 3 environments, in the central corn belt of the USA, to understand two central questions:
1) How do these traits vary over germplasm and environments?
2) How do traits relate to yield and to each other?
As expected, analyses showed a significant variability in measured phenotypic and molecular traits across both pedigrees and environments and presented a complex picture of how groups of traits interact and how they combine to produce yield. At the same time, we were able to identify genes and biological pathways underlying specific traits with supportive evidence from multiple sources. We also evaluated the opportunity to utilize automated phenotyping system that combines throughput and resolution to characterize trait variability for 52 commercially-relevant maize hybrids. Controlled environment maize phenotyping using high-resolution and high-throughput can differentiate some phenotypic traits that contribute to increased grain yield and provide and opportunity to answer specific questions under highly consistent environmental conditions.

P74

Using expression QTL with machine learning to learn about the usefulness of genomic annotations for finding causal variants

(submitted by Peter Bradbury <pjb39@cornell.edu>)
Full Author List: Bradbury, Peter J[1]; Kremling, Karl[2]; Casstevens, Terry[3]; Mejia-Guerra, Katherine[3]; Johnson, Lynn C[3]; Miller, Zachary R[3]; Zuo, Tao[3]; Buckler, Edward S[1 2 3]
[1] USDA-ARS, Robert W. Holley Center, Ithaca, NY, USA 14853
[2] Plant Breeding and Genetics Section, Cornell University, Ithaca, NY 14853
[3] Institute for Genomic Diversity, Cornell University, Ithaca, NY 14853

Simple GWAS of a dataset of gene expression levels for 280 taxa measured in several tissues using 3' RNAseq can be used to generate millions of positive associations. When the association analysis is done with site variants based on whole genome sequence, causal variants will be among the associated sites. However, many of the associated sites will simply be in LD with causal variants but not causal variants themselves. Because the associated non-causal variants are in LD with the phenotypes, they are not false positives in the statistical sense. As a result, statistical tests alone cannot be expected to identify biological false positives very effectively. By using genomic annotations with expression QTL, machine learning methods can identify additional characteristics of true positives and help identify them in the results of association tests.

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P75

Using genome-scale transposable element insertion datasets to investigate mutation in gametophytically-expressed genes

(submitted by John Fowler <fowlerj@science.oregonstate.edu>)
Full Author List: Fowler, John E[1]; Smyth, Johanna C[1]; Vejlupkova, Zuzana[1]; Wu, Shan[2]; McCarty, Donald R[2]; Koch, Karen E[2]
[1] Botany & Plant Pathology Dept., Oregon State University; Corvallis, OR USA 97331
[2] Horticultural Sciences Department, University of Florida; Gainesville, FL USA 32611

Sexual reproduction in plants involves a haploid gametophyte phase. This constrains the types of mutations that can be recovered and maintained in populations, as loss of an important gametophytically-expressed function via mutation will reduce or eliminate the ability of a gametophyte to transmit the mutation to the sporophyte. In seed plants, male and female gametophytes are developmentally distinct, and thus can be differentially sensitive to loss of function mutations (i.e., male-specific vs. female-specific transmission defects). The availability of precisely mapped insertions sites in large, transposon-mutagenized maize populations (UniformMu, the Photosynthetic Mutant Library, DsMutagenesis, and DsGFP) enables assessment of insertion frequencies in gametophyte- vs. sporophyte-expressed genes. We have constructed a database with all mapped insertion sites to conduct what we have designated as RRM (Reduced Recovery of Mutations) analysis. An initial RRM test showed expected trends: reduced representation of insertions in pollen-enriched genes in the UniformMu population (mutagenized primarily through the male), and reduced representation of insertions in embryo sac-enriched genes in the DsMutagenesis population (mutagenized solely through the female) (Chettoor et al. 2014). An updated database with expanded insertion datasets is consistent these initial observations, and further demonstrates that pollen-enriched genes defined solely by proteomic profiling (Walley et al 2016) are associated with a similar RRM trend. We also adapted Mu-Seq technology (Hunter et al. 2014) to efficiently screen individual *Mu* insertions for male transmission defects, identifying two such mutant lines from a set of twenty-four. Direct PCR genotyping using additional outcross populations confirmed that both mutations are associated with male-specific defects. This reduced appearance of deleterious mutations may help explain the higher retention rate for pollen genes of both subgenome paralogs in the maize genome, relative to seedling (sporophyte) genes.

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P76

Using RNAseq to identify key player genes driving soybean drought response in high and low nitrogen fixing genotypes

(submitted by Marianne Emery <marianneemery@mail.missouri.edu>)
Full Author List: Emery, Marianne L.[1]; Dhanapal, Arun P.[1]; King, C. Andy[2]; Purcell, Larry C.[2]; Ray, Jeffery D.[3]; Smith, James R.[3]; Elsik, Christine G.[1 4]; Fritschi, Felix B.[1]
[1] Division of Plant Sciences, University of Missouri, Columbia, MO
[2] Department of Crop, Soil, and Environmental Sciences, University of Arkansas, Fayetteville
[3] Crop Genetics Research Unit, USDA-ARS, 141 Experiment Station Road, Stoneville, MS
[4] Division of Animal Science, University of Missouri, Columbia, MO

Patterns of gene expression provide insight into plant responses to stress conditions such as drought. In legumes, the process of nitrogen (N) fixation has been shown to be sensitive to water deficit. A better understanding of the gene(s) that drive major pathway(s) involved in soybean (Glycine max) response to drought, and their effect on N fixation, will assist in the development of soybean cultivars better able to adapt in drought conditions. Here we report a comparison of two soybean cultivars, identified previously as high and low N fixing, exposed to varying levels of water availability. RNA extracted from leaf, nodule, and root tissues were sequenced using Illumina Hi-Seq (100 bp, single-end) and quantified at the gene level across environments and tissues. Gene expression values were modelled into nine clusters. Genes from each cluster were used to build a mutual information network and were further characterized. Central genes, here defined as key player genes, were identified. We believe these key player genes may be main drivers in soybean drought response and could be putative targets for improvement in future breeding efforts.

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P77

Whole plant phenotyping of maize diversity lines reveals distinct outcomes to seedling water limitation

(submitted by Nathanael Ellis <Nellis@danforthcenter.org>)
Full Author List: Ellis, Nathanael A[1]; Shakoor, Nadia[1]; Lizarraga, Cesar[1]; Mockler, Todd C.[1]; Topp, Christopher N.[1]
[1] Donald Danforth Plant Science Center; 975 N. Warson Rd. Lab 106, St. Louis, Missouri USA 63132

In light of climate change and increasing global demand for food, efforts to develop crops that thrive in non-optimal environments are essential. As the most limiting resource, varieties that yield more with less water would benefit agriculture in several ways: resilience to droughts, increased sustainability, and by expanding the areas where maize can be grown productively. Seedling establishment is critical for future plant productivity, however whole-plant responses to water limitations and the repercussions for the mature plant are poorly understood, especially at the genetic level. Our study focuses on quantifying natural variation for above- and below-ground responses to water limitation during early plant development using the maize nested association mapping (NAM) parents. Experiments were conducted using a completely automated LemnaTec Scanalyzer 3D growth and imaging system, equipped with visible (RGB) and near-infrared (NIR) imaging modalities. Water limitations were defined by field capacity: 25%, 50%, 75% and 100%, which was measured to the gram of water per day by the system. During a ten-day period spanning approximately V2-V4, plants were imaged daily to measure shoot morphology (RGB) and internal water distribution (NIR). On the final day, manual measurements were made for shoots, including biomass and leaf number. To study below-ground responses, we excavated root systems, manually measured crown root traits, and then quantified numerous root shape features using imaging and the Digital Imaging of Root Traits (DIRT) software. We found that some lines grew conservatively with reduced water, while others used as much water as was available, suggesting fundamentally different resource use strategies. To understand the effects of seedling water limitation on the mature plant, a subset of replicates was transplanted after 10 days of limited watering, then well-watered in a greenhouse until flowering. To our surprise, a handful of genotypes accumulated more biomass after experiencing seedling water restrictions than their well-watered controls. QTL mapping experiments are underway to identify the genetic basis of seedling and mature whole-plant responses.

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P78

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

(submitted by Kokulapalan Wimalanathan <kokul@iastate.edu>)
Full Author List: Wimalanathan, Kokulapalan[1 2]; Weeks, Rebecca[2 3]; Unger-Wallace, Erica[2]; Vollbrecht, Erik[1 2 3]
[1] Bioinformatics and Computational Biology, Iowa State University, Ames, IA 50011
[2] Department of Genetics Development and Cell Biology, Iowa State University, Ames, IA 50011
[3] Interdepartmental Genetics, Iowa State University, Ames, IA 50011

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.

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P79

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

(submitted by Kokulapalan Wimalanathan <kokul@iastate.edu>)
Full Author List: Wimalanathan, Kokulapalan[1 2]; Andorf, Carson[3 4]; Friedberg, Iddo[1]; Dill, Carolyn[1 2 5]
[1] Bioinformatics and Computational Biology, Iowa State University, Ames, IA 50011, USA
[2] Department of Genetics Development and Cell Biology, Iowa State University, Ames, IA 50011, USA
[3] USDA-ARS Corn Insects and Crop Genetics Research Unit, Iowa State University, Ames, IA 50011, USA
[4] Department of Computer Science, Iowa State University, Ames, IA 50011, USA
[5] Department of Agronomy, Iowa State University, Ames, IA 50011, USA

Making a genome sequence accessible and useful involves three basic steps: genome assembly, structural annotation, and functional annotation. The quality of data generated at each step influences the accuracy of inferences that can be made, with high-quality analyses producing better datasets that result in stronger hypotheses for downstream experiments. Here we report a new, high-confidence functional annotation of genes for the maize B73 reference genome. To develop this annotation set, we used sequence similarity- and protein domain-based methods as well as mixed methods developed for the Critical Assessment of Function Annotation (CAFA) competition. Individual annotation sets as well as combined outputs of multiple methods were compared both to each other, and to the existing datasets from Gramene (release 49) and Phytozome (release 12). Our new functional annotation increases the number of genes that are assigned at least one functional annotation (GO term) as well as the quality of functional assignments on average (based on F-measure). Annotations derived from the GO Annotation Methods and Evaluation (GAME) pipeline will be made accessible via MaizeGDB (http://www.maizegdb.org).

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P80

A survey of natural variation in the composition of the maize silk surface hydrocarbon metabolome

(submitted by Tes Posekany <posekany@iastate.edu>)
Full Author List: Posekany, Tes[1 2]; Qin, Wenmin[3]; Loneman, Derek[4]; Condon, Samson[3]; Lauter, Nick[1 2 5]; Nikolau, Basil J.[2 3]; Yandeau-Nelson, Marna D.[2 4]
[1] Department of Plant Pathology and Microbiology, Iowa State University, Ames, Iowa 50011
[2] Interdepartmental Genetics & Genomics Graduate Program, Iowa State University, Ames, Iowa 50011
[3] Roy J. Carver Department of Biochemistry, Biophysics & Molecular Biology, Iowa State University, Ames, Iowa 50011
[4] Department of Genetics, Development & Cell Biology, Iowa State University, Ames, Iowa 50011
[5] USDA-ARS Corn Insects and Crop Genetics Research Unit, Iowa State University, Ames, IA, 50011

Maize silks that have emerged from the protective husk leaves are exposed to many biotic and abiotic stresses (e.g. insect damage, drought, UV radiation). The extracellular silk cuticle covers the epidermis and is infused with and coated by surface lipids (SLs) that act as an environmental barrier that is comprised primarily of long-chain hydrocarbons. To begin to understand the extent of natural variation in the composition of the silk SL metabolome, we used gas chromatography-mass spectrometry (GC-MS) to profile surface hydrocarbon accumulation on husk-encased and emerged silks from 25 genetically diverse inbreds, including 20 of Nested Association Mapping (NAM) population inbred founders. We observed unbranched, non-cyclic hydrocarbons ranging in chain length from 21 to 31 carbons atoms of both even- and odd-numbered chain lengths and both saturated and unsaturated compounds. Dimethyl disulfide (DMDS) derivatizations of hydrocarbon extracts were conducted to determine the double bond positions of the alkenes, revealing two or more distinguishable structural isomers for most chain lengths that collectively reflect intricate regulatory and enzymatic complexities of desaturation chemistry. Across 20 NAM inbred founders, total hydrocarbon content varied ~5 fold on encased silks and ~6 fold on emerged silks. Accumulation of hydrocarbon constituents (e.g. nonacosane) and classes (e.g. even-numbered chain length alkanes) varies in ways not strictly associated with total accumulation, suggesting that genetic variation also affects relative composition. This study revealed that variation in surface hydrocarbon content occurs among maize inbreds, between husk-encased and emerged silks, and across years, indicating that the silk surface hydrocarbon metabolome is affected by genetic, developmental, and environmental signals. We will also discuss our silk surface hydrocarbon analysis of four ex-plant variety protection (ex-PVP) genotypes and six derived hybrids, which allows us to investigate inheritance patterns of these phenotypes and to survey natural variation that is relevant to commercial breeding programs.

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P81

A VIB platform for maize transformation and CRISPR-mediated gene editing

(submitted by Laurens Pauwels <lapau@psb.ugent.be>)
Full Author List: Aesaert, Stijn[1]; Coussens, Griet[1]; Njuguna, Elizabeth[1]; Pauwels, Laurens[1]; Van Lijsebettens, Mieke[1]
[1] VIB-UGent Center for Plant Systems Biology; Technologiepark 927; Ghent, Belgium 9052

CRISPR/Cas9 technology, with its high efficiency and simple design, has brought genome editing within reach of most molecular biologists. However, creation of gene-edited plants also relies on plant transformation and regeneration. For many crops — including maize — this forms a new bottleneck as these plant tissue culture techniques are time-consuming, laborious and often restricted to certain genotypes.
Here, we report progress on the maize transformation platform established at the VIB Center of Plant Systems Biology in Ghent, Belgium. Immature embryos of the B104 inbred line are transformed with *Agrobacterium tumefaciens* in a 8-month process yielding transgenic T1 seeds (Coussens *et al*., 2012 *J. Exp. Bot*, 63:4263–4273). Adaptations to culture media (Akoyi *et al*., 2013 *Journal of Life Science* 7(7): 677-689) and plant growth regulators allowed shortening of time in tissue culture and increasing regeneration capacity. Moreover, we report high-efficiency CRISPR/Cas9 genome editing using dual-sgRNA constructs, leading to gene deletion events and/or knocking out homologous genes.
In the US, the Iowa State University (ISU) provides researchers easy access to a maize transformation and gene-editing service (Char *et al*., 2016 *Plant Biotechnol J*. doi: 10.1111/pbi.12611). The VIB platform presented here may similarly serve the research community in Europe.

P82

Accelerated gene discovery from Setaria to maize: SvAUX1 and ZmAUX1 are required for inflorescence branch development and root gravitropism

(submitted by Hui Jiang <hjiang@danforthcenter.org>)
Full Author List: Jiang, Hui[1]; Huang, Pu[1]; Zhu, Chuanmei[1]; Barry, Kerrie[2]; Jenkins, Jerry[3]; Schmutz, Jeremy[2 3]; Box, Mathew S.[1]; Kellogg, Elizabeth A.[1]; Brutnell, Thomas P.[1]
[1] Donald Danforth Plant Science Center, 975 N Warson Rd, St. Louis, MO 63132, USA
[2] Department of Energy Joint Genome Institute, Walnut Creek, California, USA
[3] HudsonAlpha Institute for Biotechnology, Huntsville, Alabama, USA

Setaria viridis is an emerging model system for panicoid grasses due to its small size, short life cycle, good seed production, ease of crossing and transformation. To identify genes that are associated with agronomically important traits in the grasses such as panicle structure, we have developed an NMU mutagenized population consisting of ~ 20,000 M2 families. Screens of approximately 2700 families identified four mutants with sparse panicle phenotype. To fine map the gene that underlies the sparse panicle 1 (spp1) phenotype, we used bulked segregant analysis (BSA) by sequencing and mapped the gene into an interval of approximately 1Mb. Within this interval two disruptive mutations were identified in two genes. Deep resequencing of spp3 identified an independent disruptive mutation in SvAUX1, one of the two candidate genes in the ~1Mb interval, which is the only disrupted gene shared by spp1 and spp3. Complementation tests between spp1 and spp3 indicate that the mutations are allelic, thus confirming that SvAUX1 is the gene responsible for sparse panicle phenotype. Observation of early panicle development with scanning electron microscopy shows that size, shape, and position of primary branches is altered in the mutants. Synteny comparisons identified a maize ortholog of SvAUX1 and mutant characterizations confirmed a role of ZmAUX1 in inflorescence and root development. In conclusion, Setaria viridis is a tractable genetic model for rapid gene candidate identification and functional characterizations, which will accelerate functional genomics studies in maize.

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P83

An acetylated bHLH transcription factor plays an essential role in resistance of corn towards northern leaf spot

(submitted by Gaoyuan Song <sgy0097@iastate.edu>)
Full Author List: Song, Gaoyuan[1]; Yu, Mei[1]; Whitham, Steven A[1]; Walley, Justin W[1]
[1] Iowa state university, 351 Bessey, Ames, Iowa, 50011

Protein acetylation is an emerging post-translational modification that appears to play central roles during host-pathogen interactions. We are specifically investigating the role of protein acetylation in mediating the interplay between corn and the fungal pathogen Cochliobolus carbonum, the causal agent of Northern Leaf Spot. The main determinant of C. carbonum virulence is HC-toxin, which is a potent histone deacetylase inhibitor. We did an acetylome analysis, using the LC-MS/MS, on 15d old maize seedlings treated with mock, exogenous HC-Toxin as well as HC-toxin producing (TOX+) and deficient (TOX-) strains of C. carbonum. From the acetylome data, we found a bHLH transcription factor that is hyper-acetylated in HC-Toxin and C. carbonum (TOX+) treated samples, but did not observe hyper-acetylation in response to C. carbonum (TOX-). We also found more than 1,500 genes as potential targets of this bHLH using unsupervised gene regulatory network (GRN) prediction. We confirmed target genes using transcriptional reporter assays and ChIP-qPCR done in maize protoplasts. Additionally, we found, the mutations mimicking bHLH acetylation result in an enhanced activity to trigger target gene's expression, relative to wild type, in maize protoplasts. Finally, we found that silencing this bHLH, in a normally resistant corn line carrying the resistance gene Hm1, resulted in susceptibility towards C. carbonum (TOX+). These results suggested this bHLH transcription factor played an essential role in resistance of corn towards northern leaf spot.
Key words: Maize, C.Carbonum, HC-Toxin, Northern leaf spot, bHLH transcription factor, immune response.

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P84

Analysis of the A188 genome for understanding the genetic basis of maize regeneration

(submitted by Guifang Lin <guifanglin@ksu.edu>)
Full Author List: Lin, Guifang[1]; Liu, Yan[2]; Liu, Yunjun[2]; Zheng, Jun[2]; White, Frank F[3]; Wang, Guoying[2]; Liu, Sanzhen[1]
[1] Department of Plant Pathology, Kansas State University, Manhattan, KS. 66506. U.S.A.
[2] Institute of Crop Science, Chinese Academy of Agricultural Sciences, Beijing 100081, P.R.China
[3] Department of Plant Pathology, University of Florida, Gainesville, FL. 32611. U.S.A.

Genome editing technologies have made tremendous strides in recent years, spurring a need for improved transformation protocols. In maize, transformation proficiency is highly tissue- and genotype-dependent. Immature embryos from the inbred line A188 are highly regenerative and transformation-amenable, and maize lines derived from A188 are widely used for transformation. The genetic basis of regeneration in maize is poorly understood, prompting efforts to identify genetic factors underlying this trait and to apply the knowledge for improvements in transformation. In this study, sequences of A188 were generated by Illumina shotgun sequencing. A comparison between A188 and the reference line B73 revealed highly polymorphic genomes, including two megabase-level segmental duplications in A188 and pervasive presence-absence variations. HiIIA and HiIIB are stock lines used to generate robust transformable germplasm and were derived from F2 individuals of B73xA188. HiIIA and HiIIB, both of which are highly regenerable, were examined for common genomic components contributing high regeneration in conjugation with the QTL mapping results. The diversity of phenotypes between A188 and B73, including regeneration, makes the cross population valuable for the genetic analysis of many traits, and, to further facilitate the identification of genes related to regeneration and other agronomical traits, B73 and A188 double haploids (DHs) are being established. DH lines will be increased, genotyped and shared with the community. Inbred B73xA188 populations promise to be an excellent resource for leveraging genome engineering technologies and accelerating gene functional discovery in maize.

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P85

Assessing the role of two receptor-like kinases in modulating induced foliar volatile production in response to the herbivore elicitor N-linolenoyl L-glutamine

(submitted by Elly Poretsky <eporetsky@ucsd.edu>)
Full Author List: Poretsky, Elly[1]; Weckwerth, Philipp[1]; Schmelz, Eric A[1]; Huffaker, Alisa[1]
[1] University of California San Diego, 9500 Gilman Dr., La Jolla, CA, 92093-0116, USA

The fatty acid amide elicitor N-linolenoyl L-glutamine (Gln-18:3) is present in the oral secretions of Lepidopteran herbivores and is a potent inducer of vegetative volatile emissions associated with indirect defense against herbivores. Maize plants treated with Gln-18:3 emit a rich chemical blend, including numerous terpenes and green leafy volatiles (GLV). Despite the potent volatile-inducing activity of Gln-18:3, sensitivity to this elicitor varies widely among species and even among cultivars within the same species. To understand the molecular phenotype underlying plant competency to respond to Gln-18:3, better characterization of signaling components specific to the elicitor are needed. We identified two parent maize plants with differential abilities to respond to Gln-18:3, but similar responsiveness to other elicitors of volatile emissions. A recombinant inbred line (RIL) population produced from these two parents was screened for sensitivity to Gln-18:3 through measurement of induced volatile emission upon treatment. Mapping of the response patterns revealed a single quantitative trait locus (QTL) that is significantly associated with the emission of both terpene and GLVs after treatment with Gln-18:3. We have selected two candidate Receptor-Like Kinases from the genes present at this locus, and will test their potential role in modulating sensitivity to Gln-18:3 by expressing them in a heterologous system, and assessing whether these plants gain the ability to respond to Gln-18:3.

P86

Brassinosteroids and gibberellins determine growth and development through interdependent relationships

(submitted by Norman Best <nbbest@purdue.edu>)
Full Author List: Best, Norman B.[1]; Johal, Guri[2]; Dilkes, Brian P.[3]
[1] Department of Horticulture and Landscape Architecture, Purdue University, West Lafayette, IN, USA 47907
[2] Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN, USA 47907
[3] Department of Biochemistry, Purdue University, West Lafayette, IN, USA 47907

Phytohormones interact to affect maize plant height, branching, and sexual organ development. Recent cloning of the classical dwarf mutant *nana plant2* (*na2*) identified a loss-of-function mutation ortholog of *At*DWF1 required for brassinosteroid (BR) biosynthesis. We have investigated the genetic interaction between *na2* and a gibberellin (GA) biosynthetic mutant, *dwarf5* (*d5*). Both *na2* and *d5* are severe dwarfs, but have distinct additional phenotypes. Whereas *d5* tillers profusely and induces anther production in ears, *na2* has no tillers and presence of pistils in tassels (POPIT). Double mutant analyses of *na2/d5* indicate that BR and GA interact to influence plant growth dependent on the developmental context. Disruptions to BR and GA biosynthesis influence height additively, *na2* was epistatic to *d5* for tiller development, *d5* was epistatic to *na2* for POPIT, and no interaction was observed between *na2* and *d5* for anther development. Identical results were obtained by substituting with *na1* and *d1*. These mutants were also treated with chemical inhibitors of hormone biosynthesis. Treating *na2*, *d5*, and WT siblings with the BR biosynthesis inhibitor propiconazole (PCZ) or GA biosynthesis inhibitors uniconazole (UCZ) and paclobutrazol (PAC) partially phenocopied these genetic interactions. All three inhibitors were able to phenocopy the genetic results for plant height and tiller formation. PCZ treatment increased POPIT in *na2* plants but was unable to induce POPIT in *d5* or WT plants. UCZ induced anther ear in all genetic backgrounds and suppressed POPIT in *na2*. PAC did not induce anther ear in *na2* or WT siblings, nor did it suppress POPIT in *na2*. Both genetic and inhibitor studies indicate that BR and GA interactions are developmentally distinct. Comparing the genetic to the pharmacological experiments reveals the insufficiency of these chemicals to induce phenotypes from loss of hormone biosynthesis and highlights the limitations of this approach.

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P87

Characterization of a tissue-specific knockout of zap1

(submitted by Jennifer Arp <jarp2@illinois.edu>)
Full Author List: Arp, Jennifer J[1]; Moose, Stephen P[1]
[1] Department of Crop Sciences, University of Illinois; Urbana, IL, U.S. 61801

The zap1 gene is a MADS-box gene in maize within the clade containing APETELA1, which has been well-characterized for its role in inflorescence initiation and organ identity in Arabidopsis. Both zap1 and the related gene zmm15 are widely expressed in both vegetative and reproductive tissues. We found zap1 to be nitrogen responsive in vegetative tissues, and its expression increases during leaf and stem development, as the organ itself ages. Other groups have found that modulation of a related MADS-box family gene, MADS3, decreased the number of nodes in the maize stem, resulting in decreased plant height, indicating a role for this gene family in key vegetative developmental processes. To ascertain the function of zap1 specifically, a transposon insertion in the 5'UTR of zap1 was obtained in a UniformMu line. The mutant was grown in the N-responsive nursery in 2016, sampled for RNA at anthesis, and qPCR was performed to confirm expression knockout. Surprisingly, the zap1 mutant gave a tissue-specific knockout of zap1 expression only in the leaf. The zap1 mutant line retained full gene expression in ear tissues, and no floral morphology defects were detected in these plants. Alternative splicing variation did not appear to play a role in conferring differential response to transposon insertion between tissues. To characterize the genes regulated by zap1, RNAseq was performed on leaf and earshoot tissues at anthesis for plants grown under limiting and replete field nitrogen. A total of 876 genes were differentially expressed in the zap1 mutant across treatments; among those were a large number of transcription factors including EREB, WRKY, Myb, MYBR and MADS-box genes. In the leaf, zap1 may modulate expression of key transcription factors to control developmental programming during the key developmental time where the plant switches between vegetative growth and nitrogen uptake to reproductive growth and nitrogen remobilization.

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P88

Characterization of increased *Pantoea stewartii* resistance in maize *pan1* mutants

(submitted by Paula Doblas-Ibañez <pdoblasibanez@ucsd.edu>)
Full Author List: Doblas-Ibañez, Paula[1]; Deng, Kaiyue[1]; Smith, Laurie G[1]
[1] Section of Cell and Developmental Biology, University of California at San Diego, La Jolla, CA 92093, USA

Pantoea stewartii is a gram negative bacterium that is the etiological agent of Stewart's wilt, the most grave bacterial disease of maize in the Midwest and Northeast of the USA. It is a hemibiotrophic vascular pathogen that is vectored by the corn flea beetle, which introduces the bacterium into both the intercellular spaces of the leaves, where it causes water-soaked lesions (WSL), and the vasculature. *P. stewartii* preferentially colonize the xylem, leading to systemic spread throughout the plant and characteristic wilting symptoms. We have discovered that maize *pan1* mutants, that lack a leucine-rich repeat receptor-like kinase, show increased resistance to *P. stewartii*. In order to characterize the resistance phenotype, we used two different inoculation methods to separate the WSL formation (infiltration) from the xylem colonization and wilting (scratch inoculation). We found that *pan1* mutants are more resistant than the wild type to the wilting symptoms produced by *P. stewartii* plugging of the xylem after the scratch inoculation, while the formation of WSL in the apoplast is not reduced in *pan1* mutants after infiltration. Taking advantage of a GFP-tagged bacterial strain we report that *pan1* mutants are less susceptible to *P. stewartii* xylem colonization and systemic spread. Furthermore, the dissemination rate of the bacteria within the leaf xylem is impaired in *pan1* mutants. To investigate if there are differences in constitutive gene expression profiles that help to explain the enhanced resistance of *pan1* mutants we compared the transcriptomes of uninfected leaves by RNA seq. This analysis revealed that *pan1* mutants' increased resistance is not achieved via constitutive activation of plant defense. Interestingly, five genes involved in the biosynthesis of defense-related metabolites are down-regulated in *pan1* mutants. To elucidate the molecular mechanisms underlying the *pan1* mutants resistance, transcriptomic comparison of infected plants is underway, as well as metabolomic and ionomic analysis.

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P89

Characterization of metabolomic diversity of maize silk surface lipids among members of the Wisconsin Diversity Panel

(submitted by Travis Hattery <thattery@iastate.edu>)
Full Author List: Hattery, Travis J.[1]; Moore, Riley D.[2]; Loneman, Derek M.[1]; Posekany, Tes[3]; Lauter, Nick[3]; Hirsch, Candice N.[4]; Yandeau-Nelson, Marna D.[1]
[1] Department of Genetics, Development and Cell Biology, Iowa State University, Ames, IA, 50011
[2] Department of Biology, Iowa State University, Ames, IA, 50011
[3] Department of Plant Pathology and Microbiology, Iowa State University, Ames, IA, 50011
[4] Department of Agronomy and Plant Genetics, University of Minnesota, St Paul, MN, 55108

Numerous studies have been conducted to determine the functional relationships between genome, metabolome, and phenotype. Beneath a metabolic phenotype can lie a complex genetic network, and our team aims to understand the impact of structural variation in the genome (e.g., copy-number variation, presence/absence variation) on the metabolome, specifically for surface lipids. Extracellular surface lipids are specialized metabolites synthesized by epidermal cells that accumulate on aerial portions of plants. Maize silks are rich in surface lipids (e.g. hydrocarbons), which provide a protective water barrier against drought and protection against environmental stresses. However, the underlying genetic networks that organize synthesis and localization of surface lipids are not fully understood. Utilizing 500 genetically diverse inbred lines of maize that comprise the Wisconsin Diversity (WiDiv) Panel, we have extracted surface lipid metabolomes from corn silks that had emerged from encasing husk leaves, sampled in both Minnesota and Iowa during Summer 2016. Using Gas Chromatography-Mass Spectrometry (GC-MS) identification and -Flame Ionization Detector (GC-FID) quantification, the metabolomic landscapes of a genetically diverse subset of these inbred lines shows a set of approximately 50 to 60 lipid metabolites including hydrocarbons, alcohols, fatty acids, and wax esters. A high-throughput metabolomics analysis pipeline has revealed 5-fold to 30-fold variation in metabolite quantities among genotypes. In the future, genome wide association studies using the complete metabolome dataset for the WiDiv panel will lead to an increased understanding of underlying genetic networks, as well as an understanding of the extent to which structural variation in the genome can impact specialized metabolism.

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P90

Characterization of the maize hypersensitive response

(submitted by Peter Balint-Kurti <pjbalint@ncsu.edu>)
Full Author List: Balint-Kurti, Peter[1 2]; Wang, Guan-Feng[1 3]; He, Yijian[1]; Dilkes, Brian[4]; Johal, Guri[5]
[1] Dept of Entomology and Plant Pathology, NC State University, Raleigh NC 27695, USA
[2] Plant Science Research Unit, USDA-ARS, Raleigh NC, USA
[3] School of Life Science, Shandong University, China
[4] Dept of Biochemistry, Purdue University, IN 47907-2054 USA
[5] Dept of Botany and Plant Pathology, Purdue University, IN 47907-2054 USA

The hypersensitive response (HR) is a defense response found in all higher plants. It is characterized by a rapid, localized cell death of host tissue around the point of pathogen penetration and is associated a number of other physiological and transcriptional responses. So-called resistance (R-) proteins perform the dual function of recognizing the presence of a pathogen and triggering HR. We are characterizing the function of an autoactive R-protein called Rp1-D21 which triggers HR spontaneously without the presence of a pathogen. We have identified several proteins that can suppress Rp1-D21 function in an ectopic system and are now identifying loci controlling the transcriptional response to HR. We will report our latest progress.

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P91

Chromatin structure profile (MNase DNS-seq) for 15-DAP endosperm, a B73 core reference tissue

(submitted by Zachary Turpin <zmt11@my.fsu.edu>)
Full Author List: Turpin, Zachary M.[1]; Vera, Daniel L.[2]; Bass, Hank W.[1]
[1] Department of Biological Science, Florida State University, Tallahassee, FL, USA 32306-4295
[2] Center for Genomics and Personalized Medicine, The Florida State University, Tallahassee, FL, USA 32306-4295

Basic genomic processes such as gene regulation and DNA replication depend on dynamic modulation of chromatin structure. We are producing chromatin structure MNase profiles as a MaizeCODE community resource for five core reference tissues of maize B73. The epigenomic annotation comes from the Differential Nuclease Sensitivity (DNS) assay described on our project website, maizenucleosome.org. DNS-seq data marks open chromatin by measuring the biochemical accessibility of genomic loci in fixed nuclei to a diffusible enzymatic probe, Micrococcal Nuclease, MNase (Vera et al., 2015 DOI:10.1105/tpc.114.130609, and Rodgers-Melnick et al., 2016 DOI:10.1073/pnas.1525244113). The DNS-seq assay involves isolation of formaldehyde-fixed nuclei, in situ digestion with two MNase concentrations (LIGHT and HEAVY), and NGS quantification of resulting footprints. Open chromatin is localized and defined from the DIFFERENCE plots (Light - Heavy) in which positive peaks reflect MNase sensitive footprints (MSF, aka MNase HS, open chromatin) whereas negative peaks reflect MNase resistant footprints (MRF, stable nucleosomes or footprints). Given that MSFs at gene start sites are positively correlated with expression levels and that intergenic MSF sites demarcate the functional regions of the maize genome, this project will provide a foundational layer of epigenomic information for functional genomics. Data tracks and peak calls are being produced for release on Genomaize and other public browsers. Here we describe the nuclease sensitivity profile for chromatin from field-grown developing endosperm tissue harvested 15 days after pollination. Maize endosperm is agronomically important as the nutritive storage tissue for developing maize embryos, and a major source of protein, starch, and oil. It is also biologically interesting as a tissue with triploid and endoreduplicated nuclei, along with a body of literature on tissue-specific transcriptional programs. Comparative analysis of DNS-seq chromatin profiles from endosperm versus root tip will help define the chromatin structural correlates associated with differential gene expression while cataloging the intergenic functional DNA elements in maize.

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P92

Cloning and characterization of a gene which disrupts carbohydrate partitioning in *Zea mays*

(submitted by Kristen Leach <leachka@missouri.edu>)
Full Author List: Leach, Kristen A.[1]; Baker, R. Frank[1]; Brush, Parker L.[1]; Barron, Brady[1]; Wagner, Ruth[2]; Grote, Karen[2]; Peevers, Jeanette[2]; Jackson, Dave[3]; Chomet, Paul[2]; Braun, David M.[1]
[1] Division of Biological Sciences, Interdisciplinary Plant Group, Missouri Maize Center, University of Missouri, Columbia, MO 65211
[2] Monsanto, Chesterfield, MO 63017
[3] Cold Spring Harbor Lab, Cold Spring Harbor, NY 11724

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 subcellular localization of the encoded CPD6 protein and determine its function. 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.

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P93

Cloning and characterization of *Carbohydrate partitioning defective33*

(submitted by Thu Tran <tmtqk3@mail.missouri.edu>)
Full Author List: Tran, Thu M[1]; Buschmann, Tanner[1]; Bihmidine, Saadia[1]; Baker, R Frank[1]; Chomet, Paul[2]; Wagner, Ruth[2]; Grote, Karen[2]; Peevers, Jeanette[2]; Braun, David M[1]
[1] Division of Biological Sciences and Interdisciplinary Plant Group, University of Missouri-Columbia, Columbia, MO 65211 USA
[2] Monsanto, Chesterfield, MO 63017 USA

Carbohydrate partitioning is the distribution of photoassimilates from source leaves to distant parts of the plant, including the sink (importing) tissues, such as roots, developing shoots, or reproductive tissues. To identify genes controlling carbon partitioning in maize, we characterized a recessive mutant, *carbohydrate partitioning defective33* (*cpd33*), which accumulated excess starch and soluble sugars in the mature leaves. Additionally, *cpd33* mutants exhibited chlorosis and anthocyanin accumulation in the leaf blades, greatly diminished plant growth and reduced fertility. Based on their similar mutant phenotypes and from results of complementation tests, we identified five additional recessive alleles of *cpd33* (*cpd36, cpd52, cpd63, cpd82, cpd93*), all of which were generated by EMS mutagenesis. The *Cpd33* gene has been fine-mapped to a 2.5 Mb region on the long arm of chromosome 8. Whole genome sequencing analyses on separate pools of two mutant alleles, *cpd33* and *cpd36*, identified a candidate gene with a C to T transition in *cpd33*, and a G to A transition in *cpd36*. The transition mutation in *cpd33* is predicted to result in an amino acid change, and the transition mutation in *cpd36* is predicted to result in a premature stop codon. PCR amplified products of this gene from four other mutant alleles (*cpd52, cpd63, cpd82, cpd93*) were sequenced, and single nucleotide changes, which resulted in premature stop codons, were identified in all four other alleles. These results demonstrated that the *cpd33* mutant phenotype occurred because of the disruption of this gene. The *Cpd33* gene is a single-exon gene that encodes a protein of 130 kDa, which contains two predicted transmembrane domains. Interestingly, the loss-of-function mutants of the *Arabidopsis Cpd33* homolog were not reported to hyperaccumulate carbohydrates in the leaves. Ongoing studies are characterizing the molecular and physiological functions of CPD33 to understand its role in carbohydrate partitioning.

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P94

Cold maize phenotyping: Effect of seed priming on maize seedling emergence

(submitted by Gokhan Hacisalihoglu <gokhan.h@famu.edu>)
Full Author List: Hacisalihoglu, Gokhan[1]; Gustin, Jeff[2]; Kantanka, Sarfo[1]; Settles, A. Mark[2]
[1] Department of Biological Sciences, Florida A&M University, Tallahassee, FL 32307
[2] Horticultural Science Department, University of Florida, Gainesville, FL 32611

Maize is ancestrally a tropical plant and has limited cold tolerance. Cold temperature stress is a limiting factor to crop production in northern latitudes. Cold stress can occur in the soil during germination and emergence or during juvenile plant growth after initial establishment. We investigated the level of phenotypic diversity for cold tolerance in seedling emergence for twenty-seven inbred lines including all of the Nested Association Mapping (NAM) population parents. Seed priming was also tested as a possible pre-treatment to mitigate cold sensitivity. Priming treatments were explored using a synthetic solid matrix mixed together with seed under varying water and temperature conditions. All maize kernels were phenotyped with near infrared reflectance spectroscopy to measure seed density, weight, oil, protein, starch composition traits. Primed and untreated kernels were sown in cold soil, maintained for one week at the cold temperature, and shifted to optimal germination and emergence temperature. Controls without cold treatment were sown at optimal temperature for germination and emergence. A machine vision system with six DSLR cameras were developed to capture images every 30 minutes to obtain precise timing of emergence of individual seedlings. The inbred genotypes were classified as cold sensitive or tolerant based on mean emergence time and final proportion of emerged seedlings relative to optimal temperature controls. We also detected a correlation between kernel starch composition and emergence. Our results suggest that machine vision and priming are useful indices to screen and potentially improve maize cold tolerance. Importantly, sufficient variation in emergence exists in some NAM parental inbreds to enable QTL mapping of cold tolerant traits.

P95

Commercial maize hybrids and mutant genotypes reveal complex protective roles for inducible terpenoid defenses

(submitted by Shawn Christensen <shawn.christensen@ars.usda.gov>)
Full Author List: Christensen, Shawn A.[1]; Sims, James[2]; Vaughan, Martha[3]; Hunter, Charles[1]; Block, Anna[1]; Willet, Denis[1]; Alborn, Hans[1]; Huffaker, Alisa[4]; Schmelz, Eric A.[4]
[1] Chemistry Research Unit, Center for Medical, Agricultural, and Veterinary Entomology, United States Department of Agriculture–Agricultural Research Service, Gainesville, FL 32608
[2] Department of Environmental Systems Science, ETH Zurich, 8092 Zurich, Switzerland
[3] Mycotoxin Preventioni and Applied Microbiology Research, United States Department of Agriculture, Agricultural Research Service, 1815 N. University St. Peoria, IL 61604
[4] Section of Cell and Developmental Biology, University of California at San Diego, La Jolla, CA 92093-0380

Despite long-term efforts to characterize inducible biochemical defenses in maize, the presence of predominant acidic phytoalexins has only recently been established with the discovery of kauralexins and zealexins. To elucidate further defenses, we profiled terpenoids in maize tissues infected with fungi and identified a novel zealexin, termed zealexin A4 (ZA4). Evaluation of zealexins, diterpenoids, and other defense metabolites in commercial hybrid maize revealed distinct defense accumulation patterns in pathogen-infected tissues. ZA4 was strongly elicited by *Cochliobolus heterostrophus* but only weekly induced by *Fusarium graminearum* and *Colletotrichum graminicola*. ZA4 had potent antimicrobial activity against *F. graminearum*, and contrastingly, promoted *C. heterostrophus* and *C. graminicola* growth in liquid culture. Overall, a negative correlation was observed between total phytoalexin production and fungal growth. Statistical analysis supported a role for kauralexin A3 (KA3) and the drought inducible hormone abscisic acid (ABA) to have the strongest impacts on fungal growth suppression. *Anther Ear 2* mutants deficient in kauralexins demonstrated significantly improved *C. heterostrophus* and *F. verticillioides* growth compared to wild-types. Drought-induced ABA moderately reduced fungal proliferation in stems. Current results highlight the widely occurring defense functions for maize terpenoids in diverse commercial lines and highly selective activities on different fungal pathogens.

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P96

Construction of EMS-induced mutant library and identification of mutant genes in maize

(submitted by Xiujing He <hexiujing77@163.com>)
Full Author List: He1, Xiujing[1]; Pan, Chao[1]; Zheng, Qi[1]; Huang, Li[1]; Nie, Shujun[1]; Li, Hui[1]; He, Weiqiang[1]; Wang, Xuebo[1]; Wang, Bin[1]; Zhang, Li[1]; Shi, Jian[1]; Gong, Min[1]; Zhan, Jing[1]; Dai, Jie[1]; Li, Qigui[1]; Yuan, Yang[1]; Tang, Changxiao[1]; Ding, Haiping[1]; Li, Peng[1]; Zhang, Zhiming[1]
[1] Maize Research Institute, Sichuan Agricultural University, No.211 Huimin Road, Wenjiang District, Chengdu, Sichuan Province, P.R.C, 611130.

Mutagenesis library provides an extensive resource for identifying the biological functions of genes in plants. EMS mutagenesis can induce chemical modification of nucleotides, mostly single nucleotide variants, which is an efficient method to construct a mutagenesis library. Here, we employed an integrated strategy consisting of EMS mutagenesis, high-thoughout sequencing and fine mapping to identify causal mutations in maize. First, a mutagenesis library was constructed by EMS mutagenesis and phenotyping. Maize inbred line SCML203 were treated by EMS, following typical EMS mutagenesis protocol. Those individuals with interesting mutant phenotypes were isolated by screening EMS-mutagenized M2 population. Three BC1F2 populations were then constructed by crossing each mutant individual with SCML203, B73 and Mo17 inbreed line for future analysis. Moreover, we performed a deep re-sequencing of the inbred line SCML203 (~60×) to identify natural polymorphisms between SCML203 and B73 reference genome, and to develop indel markers. Finally, bulked segregant analysis by sequencing (BSA-seq), bulked segregant RNA-Seq (BSR-Seq) and fine mapping were performed to detected the causal mutation at whole-genome level. Using the above strategy, we successfully identified a leaf-color gene lc1 within 107 kb region on chromosome 6, which was predicted to participate in rRNA processing. EMS-induced G->A transition resulting in protein sequence change was detected in the coding region, and further confirmed by RNA-seq analysis.

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P97

Contribution of translational control to establishing the distinct proteomes of bundle sheath and mesophyll cells in maize

(submitted by Nicholas Stiffler <nstiffle@uoregon.edu>)
Full Author List: Chotewutmontri, Prakitchai[1]; Stiffler, Nicholas[1]; Kumar, Indrajit[2]; Brutnell, Thomas[2]; Barkan, Alice[1]
[1] Institute of Molecular Biology, University of Oregon; Eugene, OR, USA 97403
[2] Donald Danforth Plant Science Center; St. Louis, Mo, USA 63132

C4 photosynthesis in maize occurs by partitioning photosynthesis between two morphologically and functionally-distinct cell types, bundle sheath (BS) and mesophyll (M) cells. The distinct proteomes of BS and M cells (Majeran et al, Plant Cell 2010) result in part from distinct transcriptomes (Li et al Nat Gen 2010; Tausta et al, J Exp Bot 2014). However, little attention has been paid to the possibility that translational regulation contributes as well. We are using ribosome profiling to provide a genome-wide view of the contribution of differential translation to establishing the distinct proteomes of BS and M cells. Ribosome profiling uses deep-sequencing to map ribosome footprints on mRNAs. Normalization of ribosome footprint abundance to RNA-seq data is used to infer translational efficiencies. We established a rapid mechanical fractionation procedure that results in highly enriched BS and M fractions within minutes of tissue harvest. RNA-seq data from these fractions correlates well with those reported for BS and M fractions recovered by laser capture microdissection (Tausta et al, 2014). We found that differential expression of chloroplast genes in BS and M cells results primarily from differences in mRNA abundance, but differences in translational efficiency amplify mRNA-level effects in some instances (Chotewutmontri, Barkan, PLOS Gen 2016). Analysis of the cytosolic data is in progress, but results so far suggest that differences in translational efficiency contribute to the differential expression of roughly half of the genes whose translational output differs by > 2-fold in BS and M cells. In some cases, differences in translational efficiency amplify the effects of differential RNA accumulation, whereas in others, differences in translational efficiency dominate. Differences in mRNA abundance are buffered by the opposite change in translational efficiency for a small set of genes. We are currently seeking biological correlations with these distinct behaviors and clues as to the underlying mechanisms.

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P98

Control of leaf angle1 (Cla1) regulates leaf architecture in elite breeding materials of maize

(submitted by Yingying Cao <ycao@danforthcenter.org>)
Full Author List: Cao, Yingying[1]; Ku, Lixia[1]; Guo, Shulei[1]; Ren, Zhenzhen[1]; Li, Pinghua[1]; Tian, Feng[2]; Lai, Jinsheng[2]; Chen, Yanhui[1]; Brutnell, Thomas P.[3]
[1] College of Agronomy, Synergetic Innovation Center of Henan Grain Crops and National Key Laboratory of Wheat and Maize Crop Science, Henan Agricultural University, 95 Wenhua Road, Zhengzhou, 450002, China
[2] National Maize Improvement Center of China, China Agricultural University, Yuanmingyuan Western Road, Beijing, 100193, China
[3] Donald Danforth Plant Science Center, 975 North Warson Road, St. Louis, MO 63132, USA

As one of the world's most important crops, maize yield improvements are critical for global food security. Maize yield have increased dramatically in the last several decades from both improved agronomic practices and selection by breeders for higher planting densities. The selection of upright leaf architecture enables plants to be grown at higher density while minimizing the shading of neighboring plants and increasing the efficiency of light capture. To identify genetic components of leaf architecture important for yield gains, we took advantage of a recombinant inbred line population developed from two elite lines, Shen137 and Yu82 that have contributed significantly to hybrid seed production in China. Fine mapping of a major effect QTL for leaf angle led to the identification of *Control of leaf angle 1 (Cla1)*, a novel component of the leaf angle genetic program. Genome wide association studies (GWAS) confirmed the contribution of *Cla1* in the control of leaf angle across a diverse germplasm collection. Interaction of CLA1 with ZmIAA17 suggests that *Cla1* regulates the extent of leaf auricle development through an auxin signaling cascade. These results suggest several new candidates for the manipulation of leaf architecture in maize in elite breeding materials to fine tune photosynthetic performance under increasing planting densities.

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P99

Deciphering the role of Source-Sink Cross-Talk in regulation of Monocarpic Senescence

(submitted by Rajandeep Sekhon <sekhon@clemson.edu>)
Full Author List: Yi, Jakyung[1]; Saski, Christopher[2]; Kresovich, Stephen[2]; de Leon, Natalia[3 4]; Kaeppler, Shawn[3 4]; Sekhon, Rajandeep[1]
[1] Department of Genetics and Biochemistry, Clemson University, Clemson, SC, USA
[2] Institute for Translational Genomics, Clemson University, Clemson, SC, USA
[3] Department of Agronomy, University of Wisconsin, Madison, WI, USA
[4] DOE Great Lakes Bioenergy Research Center, Madison, WI, USA

Monocarpic senescence in annual crops reduces photosynthetic assimilation and limits the duration of carbon-capture phase thus resulting in a loss of net carbon yield. The onset of senescence is profoundly affected by source-sink interactions but the underlying molecular mechanisms are not well understood. In maize, senescence is prematurely induced in plants lacking primary (grain) sink likely signaled by sugars accumulating in the leaves. We have screened natural diversity and identified genetic differences for the ability of plants to avoid the premature senescence by translocating sugars to stalks. This analysis also identified tremendous genetic variation for natural senescence and stay-green. QTL underlying premature and natural senescence were identified in a bi-parental population and a diversity panel. We discovered QTL that appeared both for premature and natural senescence indicating that these two processes share common underlying molecular mechanisms. We have corroborated the QTL effect in near isogenic lines (NILs) have and begun fine mapping QTL in these NILs. Sorghum also has tremendous variation for sugar partitioning with sweet genotypes accumulating most of the carbon as sucrose in stalks while grain genotypes primarily accumulating sugars in the grain. Comparative genomic analysis of the maize QTL in BTX623 (grain) and Rio (sweet) sorghum inbred lines revealed tremendous synteny and interesting copy number variation the sorghum lines. This observation indicates certain mechanistic similarities for sugar partitioning in these species. We are analyzing candidate genes in these syntenic regions *in silico* to identify potentially causal sequence variants that may explain differences in sugar partitioning in sorghum and maize. Transcriptional analysis of possible candidate genes including those involved in sugar transport and signaling in maize genotypes differing for their ability to translocate sugars in stem will be presented. We are now analyzing the Mu mutants in maize and T-DNA mutants in *Arabidopsis*.

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P100

Defining the SUMOylation system in *Zea mays* and its roles in seed development and stress protection

(submitted by Robert Augustine <raugustine@wustl.edu>)
Full Author List: Augustine, Robert C.[1 2]; Walker, Joseph[2]; York, Samuel S.[1 2]; Rytz, Thérèse C.[1 2]; Mahoy, Jill[3]; Dorris, Blake[2]; Kaeppler, Heidi F.[3]; Vierstra, Richard D.[1 2]
[1] Washington University in St. Louis, Department of Biology, St. Louis, MO, USA 63130
[2] University of Wisconsin - Madison, Department of Genetics, Madison, WI, USA 53706
[3] University of Wisconsin - Madison, Department of Agronomy, Madison, WI, USA 53706

Plants mount a variety of defense responses to mitigate damage inflicted by environmental stress. Among the fastest is the rapid and reversible conjugation of small ubiquitin-related modifer (SUMO) to an array of mostly nuclear proteins, particularly those directing chromatin modifications and RNA processing. Despite its established importance in stress tolerance, little is known about how SUMOylation confers this protection, especially in crop species. Using maize as a model, we identified the suite of genes encoding the maize SUMO system and uncovered both conserved and cereal-specific components by phylogenetic analyses. RNA-Seq profiling revealed that core components of the SUMO machinery are ubiquitously expressed. Nonetheless, a majority of SUMO pathway components are also transcriptionally upregulated in a defined temporal manner within the maturing endosperm, which coincides with elevated SUMO conjugate accumulation in this tissue. In addition, robust stress-induced SUMOylation is triggered within minutes in seedlings exposed to heat and oxidative stresses. To identify these developmentally-stimulated and stress-induced SUMO conjugates, we exploited proteomic approaches combined with transgenic maize lines expressing variants of 6His-tagged SUMO1 that would enable efficient target purification along with the global identification of SUMO binding sites. Preliminary mass spectrometric analyses with heat-stressed maize seedlings identified >200 SUMOylated proteins, including RNA metabolism/splicing factors, ribosomal subunits, sucrose synthase1, chromatin modifiers, transcription factors including members of the indeterminate-domain protein family, and SUMO and ubiquitin proteasome system components. Introgression of the SUMO purification lines into SUMO mutants such as *mms21-1*, which we found exhibits pleiotropic growth defects including dwarfism and male sterility, will now enable functional analyses and the assignment of SUMOylation targets to their respective SUMO conjugation/deconjugation machineries. Taken together, these studies lay the groundwork for defining the maize SUMO system, identifying SUMOylation targets, and providing reverse-genetic tools needed to highlight the importance of this modifier during maize development and stress protection.

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P101

Deletion of an endoplasmic reticulum stress response element in a ZmPP2CA gene facilitates drought tolerance of maize seedlings

(submitted by Yanli Xiang <387716984@qq.com>)
Full Author List: Xiang, Yanli[1]; Sun, Xiaopeng[1]; Dai, Mingqiu[1]
[1] National Key Laboratory of Crop Genetic; No.1,Shizishan Street, Hongshan District; Wuhan; Hubei Province; P.R.China; 430070

Drought is a major abiotic stress that causes the yearly yield loss of maize, a crop cultured worldwide, thus breeding drought tolerant maize cultivars is a priority target of the world agriculture. Clade A PP2C phosphatases (PP2CA) are conserved in plants and are important for ABA signaling and plant drought response. However, the natural variations of PP2CA genes that directly associated with levels of drought tolerance remain to be elucidated. Here, we conducted a candidate gene association analysis of ZmPP2CA gene family in a maize panel consisting of 368 varieties collected worldwide, and identified a drought responsive gene ZmPP2CA10 that is tightly associated with drought tolerance. We found that the degrees of drought tolerance of maize cultivars negatively correlate with the expression levels of ZmPP2CA10. ZmPP2CA10, like its Arabidopsis orthologs, interacts with ZmPYL ABA receptors and ZmSnRK2 kinases, suggesting that ZmPP2CA10 functions in mediating ABA signaling in maize. Transgenic studies in maize and Arabidopsis confirmed that ZmPP2CA10 plays a negative role in regulating drought tolerance. Further, a causal natural variation, InDel-338 (causing a deletion of ERSE, Endoplasmic Reticulum Stress response Element) in the 5'UTR region of ZmPP2CA10 was detected, and this deletion causes loss of ER stress- induced expression of ZmPP2CA10, leading to increased plant drought tolerance. Our findings provide direct evidence linking ER stress signaling with drought tolerance, and the results from this study provide genetic recourses that can be directly used in breeding of drought tolerant maize cultivars.

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P102

Detection of *in planta* ZFN activity and chimerism

(submitted by Stephen Novak <snnovak@dow.com>)
Full Author List: Novak, Stephen[1]; Lee, Ryan M[1]; Worden, Andrew A[1]; Skaggs, Nicole K[1]; Chen, Wei[1]
[1] Dow AgroSciences LLC 9330 Zionsville Road Indianapolis, IN 46268

In planta gene targeting, via double strand breaks (DSB) using site specific nucleases has become increasingly attractive for trait product development due to its decreased reliance on efficient plant transformation. However, DSB-induced gene targeting through breeding crosses often results in chimeric F1 tissues. Reporter genes, such as GUS or GFP, combined with histochemical staining have frequently been used to visualize the mutations as a proof of concept. However, for commercial trait product development, these reporter gene systems may not be suitable. Here we report the use of a simple quantitative PCR based method to detect the activity of zinc finger nucleases (ZFN). Each ZFN recognizes only one DNA binding site and creates one DSB. During the process of DSB repair, the plant cells can make small deletions and/or insertions at the site of the DSB. As a measurement of relative ZFN activity, qPCR based disruption assays were used to detect imperfect repair. Spatial/temporal differences in somatic and meristem tissue were also detected using this methodology.

P103

Developing a robust pipeline for genome editing in the Illinois Long Term Selection experiment

(submitted by Stephen Jinga <sjinga2@illinois.edu>)
Full Author List: Jinga, Stephen J.[1]; Rhodes, Brian H.[1]; Moose, Stephen P.[1]
[1] Department of Crop Sciences; University of Illinois, Urbana-Champaign; Urbana, Illinois, 61801

The Illinois Long Term Selection Experiment is a unique genetic resource for identifying and characterizing genes selected for nitrogen use and protein accumulation in maize. To facilitate study of gene functions, we aim to establish a CRISPR Cas9 mediated genome-editing system in these novel genetic backgrounds. A media regime has been developed for successfully regenerating fertile plants of both Illinois High Protein (IHP) and Illinois Low Protein (ILP). Currently, putative transgenic lines expressing the Cas9 protein have been recovered using NPTII as a selectable marker. These Cas9 positive lines will be used to make targeted mutations with this germplasm. We have also initiated experiments to edit the Prolamin Box Binding Factor (PBF), which regulates zein gene expression and shows changes in both allele frequencies and mRNA expression that are consistent with PBF being a target of selection for grain protein concentration. In addition to generating knockout mutations, we are also investigating the functional significance of variation in the length of an asparagine (Asn) repeat motif found at the C-terminus of PBF. This Asn repeat shares features with triplet repeat expansions studied in Arabidopsis and trinucleotide repeat disorders in humans such as Huntington's disease. It is hypothesized that variation in the Asn rich region of PBF could act as a sensor to control α-zein accumulation in response to incoming supply of amino acids, or possibly interacting with other transcription factors such as opaque-2. To target this Asn-repeat motif, single-guide RNAs were designed to create variation in Asn repeat length in conjunction with expressed Cas9. Guide RNA constructs will be introduced via biolistics and screened by Sanger sequencing to confirm targeted edits in the genome.

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P104

Developing the enviratron: A facility for automated plant phenotyping under varied growing conditions

(submitted by Carolyn Lawrence-Dill <triffid@iastate.edu>)
Full Author List: Whitham, Steven[1]; Howell, Stephen[1]; Lawrence-Dill, Carolyn J.[1]; Lubberstedt, Thomas[1]; Tang, Lie[1]
[1] Iowa State University

The Enviratron is a new concept and design in plant phenotyping with the purpose of testing plant performance under different environmental conditions. In contrast to current phenotyping facilities, the Enviratron is designed to analyze plant growth and performance under up to eight different environmental conditions in one experiment. Also unlike current phenotyping facilities, plants are not conveyed to a central analyzing station, instead a mobile robotic analyzer (rover) equipped with a sensor array visits plants, minimizing disturbances in the growth environment. In analyzing the relationship between G X E (genotype by environment), most phenotyping facilities are equipped to vary G. The Enviratron's special feature is its capacity to vary E. The Enviratron consists of eight growth chambers that each can be programmed to a unique environment that can vary slightly in a single parameter or by different climate scenarios, including temperature, CO2, humidity, water, and light (duration and intensity). The rover's sensor array includes a holographic camera, hyperspectral sensor, fluorescence detector, infrared detector, and Raman scattering spectrometer. Individual pots are designed to have soil water potential sensors with a watering system to maintain chosen soil water potentials. To enable downstream discovery and reuse of diverse data collected using the Enviratron system, a MIAPPE-compliant metadata collection and reporting mechanism is under development. Learn more about Enviratron online at http://enviratron.iastate.edu.

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P105

Discovery and characterization of a β-selinene derived terpenoid phytoalexin pathway in maize (*Zea mays*)

(submitted by Yezhang Ding <yeding@ucsd.edu>)
Full Author List: Ding, Yezhang[1]; Huffaker, Alisa[1]; Köllner, Tobias G.[2]; Weckwerth, Philipp[1]; Spencer, Joseph L.[3]; Lipka, Alexander[4]; Schmelz, Eric A.[1]
[1] Section of Cell and Developmental Biology, University of California at San Diego, La Jolla, CA 92093-0380
[2] Department of Biochemistry, Max Planck Institute for Chemical Ecology Hans-Knöll-Straße 8, D-07745 Jena
[3] Illinois Natural History Survey, University of Illinois, Champaign, IL 61820
[4] Department of Crop Sciences, University of Illinois, Urbana, IL 61801

Maize (*Zea mays*) produces a diverse suite of protective biochemicals following herbivore and pathogen attack. Among the well-studied pathways, complex volatile terpenes have been genetically, biochemically and ecologically proven to function as indirect defenses mediating the attraction of natural enemies. In contrast, the diversity, origin and function of non-volatile terpenoids in maize remains poorly understood. To uncover additional maize defenses, metabolomic profiling was conducted on the maize field roots exposed to natural combinations of herbivore and pathogen challenge. Unexpected and surprisingly high levels of eudesmane sesquiterpenoids, including β-selinene and β-costic acid dominated the chemical profiles in the field root samples. While not previously reported in maize, members of the Asteraceae family containing high levels of β-costic acid have been long utilized for potent antibiotic activities against diverse organisms. To define the gene(s) responsible for maize β-costic acid biosynthesis, we utilized combined genetic mapping approaches including the Intermated B73 × Mo17 (IBM) population of recombinant inbred lines (RILs), IBM near-isogenic lines (NILs), and the Goodman diversity panel for GWAS to collectively identify terpene synthase 21 (*ZmTps21*) as the single gene responsible for β-selinene biosynthesis that is essential for β-costic acid production. Biochemical characterization of ZmTps21 in *E. coli* confirmed that the functional Mo17 *ZmTps21* allele encodes a highly specific β-selinene synthase. Consistent with phytoalexin biosynthetic pathways, *ZmTps21* transcripts strongly accumulate following challenges with a wide range of fungal pathogens. As a significant insect pest of U.S. maize production, western corn rootworm (WCR, *Diabrotica virgifera virgifera*) larvae also promotes the accumulation of ZmTps21 products. Importantly, concentrations of β-costic acid well below those detected in field grown maize roots strongly inhibited the growth of all maize fungal pathogens tested in vitro. Thus, ZmTps21, β-selinene, β-costic acid are major components of a previously unrecognized maize defense pathway that contribute to crop protection under combined biotic stresses.

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P106

Dissecting the role of a maize *LysMe* gene in arbuscular mycorrhizal symbiosis using *Ac/Ds* mutagenesis

(submitted by Quan Zhang <qzhang@danforthcenter.org>)
Full Author List: Zhang, Quan[1]; Rong, Ying[1]; Brutnell, Thomas[1]
[1] Donald Danforth Plant Science Center; 975 N. Warson Rd., St. Louis, MO, USA 63132

Arbuscular Mycorrhizal (AM) symbiosis is one of the most ancient plant-microbe interactions established. In AM symbiosis, fungus infection is preceded by mutual recognition of both symbiotic partners and the activation of a downstream signaling pathway in plants. Although some of the components of mycorryhizal colonization are shared with pathogen-associated molecular pattern (PAMP) triggered immunity (PTI), it is unclear how the specificity in response is maintained. In this study a *LysMe* gene is shown to be strongly induced early during mycorrhizal symbiosis, in both maize and *Setaria viridis* , a model panicoid grass. *ZmLysMe* encodes a small membrane protein containing a single LysM motif. A genome scan shows that this gene is present only in plants capable of forming AM symbiosis, but absent in non-mycorrhizal plant species, including *Arabidopsis thaliana*. To characterize gene function, we mobilized a *Ds* donor located 80 kb away from the gene target. Two *ZmLysMe Ds* insertion alleles were identified through PCR-based screens of 12,000 *Ac/Ds* F1 plants, using a high throughput planting and genotyping platform. Homozygous *ZmLysMe* mutants were inoculated with *Rhizophagus irregularis* and plants were analyzed for the mycorrhizal colonization, however normal mycorrhizal colonization and arbuscule formation was observed. Another *Ac/Ds* mutagenesis of a closely related *ZmLysMe* gene is underway to examine the possibility of the gene duplication and redundancy in the maize genome.

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P107

Dissection of contributions by *Ndpk1* to sugar and oxygen responses in maize

(submitted by Maria Angelica Sanclemente <sanangelma@ufl.edu>)
Full Author List: Sanclemente, Maria Angelica[1]; DiMare, Adriana[1]; Singh, Jugpreet[2]; Ma, Fangfang[1]; Koch, Karen[1 2]
[1] University of Florida, Horticultural Sciences Department, P.O. Box 110690, Gainesville, FL 32611-0180
[2] University of Florida, Plant Molecular and Cellular Biology, P.O. Box 110690, Gainesville, FL 32611-0180

Nucleoside diphosphate kinases (NDPKs) have dual roles in metabolism and signaling. These multifunctional enzymes maintain balanced ratios of nucleoside triphosphates and diphosphates (e.g. ATP/ADP, UTP/UDP). Our initial appraisal of *Ndpk1* action using a maize root-tip system indicated clear responses to both sugar abundance and low O_2. Moreover, these effects were rapid, with elevated levels of *Ndpk1* mRNA evident within 3 to 6h. To determine the impact of these and other actions of *Ndpk1*, we compared contributions in wild-type and *ndpk1*-mutant maize. A Mu-transposon in the (5'UTR) of *Ndpk1* reduced mRNA levels for this gene to 20% of wild-type and enzyme activity to 57%. First, we tested oxygen and sugar responsiveness of *Ndpk1* in mutant and wild-type root tips. When sugar (2% glucose) and oxygen conditions (0.2% O_2) were controlled in an excised-root-tip system, levels of *Ndpk1* mRNA rose more rapidly in the knock-down mutant, and were evident within 3h. Neither oxygen nor sugar responsiveness was thus impaired in mutant material. Second, to identify relationships in transcript regulation, we analyzed wild-type and mutant root tips by RNAseq after exposure to different sugar levels under low O_2. The number of differentially expressed genes after 9h of hypoxia in the presence of glucose was markedly reduced in the *ndpk1* mutant root tips. Moreover, up-regulation of ribosome-related genes was inhibited. Third, phenotypic appraisal showed that mutant seeds germinated more slowly, consistently lagging behind by approximately 1d compared to wild-type seeds. Metabolite analysis showed that after germination, mutant root tips had significantly lower levels of glucose, fructose, and sucrose. Collective results indicate that roles of *Ndpk1* extend beyond germination and include direct or indirect modulation of translation under low-oxygen stress as well as sugar and energy metabolism.

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P108

EMB-7L is required for embryogenesis and plastid development in maize through participation in RNA splicing of multiple chloroplast genes

(submitted by Ningning Yuan <nnyuan@sibs.ac.cn>)
Full Author List: Yuan, Ningning[1]; Liu, Hongjun[1]; Wang, Jiechen[1]; Wang, Wenqin[2]; Wu, Yongrui[1]
[1] National Key Laboratory of Plant Molecular Genetics, CAS Center for Excellence in Molecular Plant Sciences, Institute of Plant Physiology & Ecology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai 200032, China
[2] College of Agriculture and Biology, Shanghai Jiaotong University, Shanghai 200240, China

Embryo and endosperm originate from the double fertilization but have different developmental fates and biological functions. Partially for this reason, they have been actively studied and compared in differential gene expression. Many mutants have been identified to specifically affect the development of embryo and endosperm. We identified a maize seed mutant (*embryo specific, emb*), whose embryo is far more severely affected than the endosperm. In the W22 background, the embryo of *emb* arrests at the transition stage while the endosperm appears nearly normal in size compared to the wild type (WT). At maturity, the embryo in W22-*emb* is very small or even couldn't be seen, but the endosperm is able to develop into comparably a WT' size. The accumulation of zeins and starch in the emb endosperm are not apparently affected. Although *emb* in W22 is lethal, a few of *emb* from the F2 population of W22-*emb* and B73 can germinate and grow albino seedlings, in which chloroplasts rarely contain the thylakoids and starch grains. We cloned the mutant gene on the Chromosome 7L and renamed it as *emb-7L*. *Emb-7L* encodes a chloroplast-localized P-type Pentatricopeptide Repeat (PPR) protein. *Emb-7L* is generally expressed in all tissues examined, but it has a relatively higher expression in leaves. P-type PPR proteins have functions in splicing of the pre-mRNAs. Indeed, RT-PCR assays demonstrated that the efficiency of the splicing of NdhA, NdhB, AtpF and Rpl2 pre-mRNAs was dramatically decreased in the *emb-7L* albino seedling leaves; in the *emb-7L* endosperm, only AtpF and Rpl2 but not NdhA and NdhB were affected in their pre-mRNA splicing. Our study suggests that the generally expressed EMB-7L has tissue specificity in pre-mRNA splicing of chloroplast genes, which might cause the differential developmental defects in the *emb-7L* embryo and endosperm.

P109

Engineering amyloplast 6-phosphogluconate dehydrogenase activity to improve heat stability of the oxidative pentose phosphate pathway

(submitted by Camila Ribeiro <camila.ribeiro@ufl.edu>)
Full Author List: Ribeiro, Camila[2]; Myers, Alan M.[3]; Hennen-Bierwagen, Tracie[3]; Cline, Kenneth C.[1 2]; Tracy, William F.[4]; Boehlein, Susan D[1]; Hannah, L. Curtis[1 2]; Settles, A. Mark[1 2]
[1] Horticultural Sciences Department, University of Florida, Gainesville, Florida, 32611
[2] Plant Molecular and Cellular Biology Program, University of Florida, Gainesville, FL, 32611
[3] Department of Biochemistry, Biophysics and Molecular Biology, Iowa State University, Ames, Iowa, 50011
[4] Department of Agronomy, University of Wisconsin, Madison, Wisconsin, 53706

Heat stress reduces maize grain weight and quality. Starch synthesis in the endosperm is sensitive to high temperature stress and has the potential to be a limiting pathway for grain yield under heat stress. In addition to enzymes directly involved in starch biosynthesis, chloroplast-localized 6-phosphogluconate dehydrogenase (PGD3) is critical for starch accumulation. PGD3 is one of three enzymes in the oxidative section of the Pentose Phosphate Pathway (PPP). Maize encodes two cytosolic isozymes, PGD1 and PGD2. Double mutants of *pgd1; pgd2* have a nearly complete loss of cytosolic activity and develop normal kernels. We compared endosperm enzyme activity from the pgd3 mutant and *pgd1; pgd2* double mutants.Cytosolic PGD1 and PGD2 isozymes are heat stable, while the amyloplast-localized PGD3 is heat labile under in vitro and in vivo heat stress conditions. To develop a heat stable 6-phosphogluconate dehydrogenase localized to amyloplasts, we developed constructs to fuse the *waxy1* N-terminal chloroplast targeting sequence to the Pgd1 and Pgd2 open reading frames. The W::PGD1 and W::PGD2 fusion proteins import into isolated pea chloroplasts indicating that the targeting sequence is functional. Transgenic maize plants were generated to express W::PGD1 and W::PGD2 under the 27 kDa γ-zein promoter to confer endosperm specific expression. Transformants have increased 6-phosphogluconate dehydrogenase enzyme activity and isozyme activity assays suggest the increase is due to higher levels of PGD1 and PGD2. Transgenic endosperm shows enhanced heat stability in vitro. The W::PGD1 and W::PGD2 transgenes complement the pgd3 defective kernel phenotype suggesting the fusion proteins are targeted to the amyloplast. A preliminary field experiment suggests the W:PGD1 transgene can mitigate grain yield losses in heat stressed conditions. These data support a model in which the amyloplast PPP contributes to maize yield loss during heat stress.

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P110

Evidence for site-specific transformation in maize using PhiC31 integrase

(submitted by Nathaniel Graham <ndgraham@mail.missouri.edu>)
Full Author List: Graham, Nat D[1]; Swyers, Nathan C[1]; Cody, Jon P[1]; Zhao, Changzeng[1]; Birchler, James A[1]
[1] University of Missouri; Columbia, Mo

Genetic engineering has become an indispensable tool in the development of new maize lines, both in the industry sector and academia. Despite this, little progress has been made in improving the transformation process, which is time-consuming and expensive. One of the explanations for this inefficiency is there is no control over where in the genome the transgene will be inserted. To combat this problem, we have inserted the unidirectional, irreversible recombinase phiC31 integrase under the control of a ubiquitin promoter into maize line HiII, as well as its corresponding recombination sites. These integrated attachment sites are designed to accept future additional transgenes at these genomic locations with continued stacking. Using a GUS reporter that itself carries recombination sites in such a configuration that recombination will activate it, the function of phiC31, as well as the recombination sites, were shown to be functional. This was demonstrated both transiently and with the recombinase inserted within the genome. Additionally, using a previously integrated recombination site located behind a ubiquitin promoter, we were able to insert biolistically a promoterless GUS reporter carrying a complementary recombination site into the genomically integrated site and detect GUS signal, which is not found in the absence of integrase or the genomic attachment site. Furthermore, the recombinase phiC31 excisionase, which can unidirectionally reverse integrase recombination, was also shown to be transiently functional in maize. These results demonstrate that the phiC31 integrase system is fully functional in maize and can be utilized for site-specific maize transformation.

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P111

Expansion of maize genomics, genetic engineering and gene editing research and service capacity in the U.S. via the Wisconsin Crop Innovation Center

(submitted by Heidi Kaeppler <hfkaeppl@wisc.edu>)
Full Author List: Kaeppler, Heidi F.[1]; Petersen, Michael[1]; Lor, Vai S.[1]; Miyamoto, Amy[1]; Martinell, Brian[1]; Mahoy, Jill A.[1]; Kaeppler, Shawn M.[1]
[1] Uiversity of Wisconsin, Madison, Wisconsin, USA 53706

Genetic engineering and gene editing systems are critical components in the advancement of maize functional genomics and epigenomics research, and genetic crop improvement efforts. While substantial improvements in the areas of DNA sequencing, epigenome interrogation, bioinformatics, and high throughput phenotyping have led to continuous increases in the types and numbers of sequences identified for characterization and testing in maize genetic research and crop improvement applications, advances in genetic engineering techniques have lagged, creating a substantial bottleneck in maize translational genomics investigation. This bottleneck consists of both biological and efficiency limitations of current public maize tissue culture and transformation techniques, and overall lack of capacity on the national level. Recently, the Wisconsin Crop Innovation Center was established 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. WCIC was initiated to dramatically increase U.S. research capacity and is intended to work together with other public crop biotechnology facilities to enhance research capacity and accelerate public agricultural research outputs. Current maize-related research and service activities underway at WCIC include genetic investigation of genotype-dependent tissue culture response in maize, development of novel, transformable maize germplasm, investigation and implementation of high efficiency genotype-independent transformation and gene editing protocols, and automation of transformation protocols and LIMS development for high throughput production. WCIC seeks collaborations with partners that have need for large-scale projects. Initial fee-for-service rates for specific services are available on the WCIC website (cropinnovation.cals.wisc.edu), 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.

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P112

Fine mapping and characterization of genes controlling maize nitrogen utilization efficiency

(submitted by Brian Rhodes <bhrhode2@illinois.edu>)
Full Author List: Rhodes, Brian H[1]; Liu, Yuhe[1]; Nichols, Devin M[1]; Moose, Stephen P[1]
[1] University of Illinois, 1201 West Gregory Drive, Urbana, IL, 61801

An important component to increasing crop productivity is improving Nitrogen Utilization Efficiency (NUtE). In maize this trait is measured as the ratio of grain yield to accumulated plant N. Enhancing NUtE offers substantial economic and environmental benefits, but little is known about the genetic mechanisms that govern variation for NUtE within maize populations. Our group has conducted high density genetic mapping for NUtE in a hybrid population developed from the intermated B73 X Mo17 recombinant inbred lines (IBMRILs), test crossed to the Illinois High Protein 1 (IHP1) inbred line, which has a superior capacity for N uptake but low NUtE. We identified 9 robust strong effect QTL for NUtE that range in size from 14-9030 kbp and aim to identify causal genetic variants. The largest effect QTL is localized to a 420 kbp region on chromosome 1 containing 10 annotated genes, including a cluster of 5 beta-expansin genes. Expansins contribute to cell enlargement through the loosening of the cell wall and thus are promising candidates for promoting increased growth in response to N. A second QTL for grain nitrogen/protein concentration has been localized to a single candidate gene that likely regulates autophagy, a process important for nitrogen remobilization. In addition to analysis of mutant alleles and near-isogenic lines for the QTL interval, we have created transgenic maize inbred lines with altered expression of this candidate gene, each of which modify grain protein concentration in both the inbred background and in F1 ears following hybridization. Genome editing experiments are in progress to further verify gene function for candidate genes within our NutE regions. The results of this project will aid the development of maize hybrids that require lower nitrogen inputs and therefore would reduce costs for farmers and mitigate environmental and health effects associated with high ambient nitrogen levels.

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P113

Fine mapping of a modifier of *Oy1*, a modulator of chlorophyll biosynthesis and plant vigor

(submitted by Rajdeep Khangura <rkhangur@purdue.edu>)
Full Author List: Khangura, Rajdeep S[1]; Venkata, Bala P[3]; Marla, Sandeep R[4]; Dilkes, Brian P[2]; Johal, Gurmukh S[1]
[1] Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN 47907
[2] Department of Biochemistry, Purdue University, West Lafayette, IN 47907
[3] Donald Danforth Plant Science Centre, St. Louis, MO 63132
[4] Department of Agronomy, Kansas State University, Manhattan, KS 66506

Maize exhibits tremendous genetic diversity for various qualitative and quantitative traits. This natural variation can be tapped using MAGIC (Mutant-Assisted Gene Identification and Characterization), a novel enhancer/suppressor screen that relies on standing variation that exists naturally in the germplasm. To reveal variation that may be used to improve photosynthesis, we conducted a MAGIC screen using *Oy1*, which is a semi-dominant mutant of magnesium chelatase that catalyzes the first committed step of chlorophyll biosynthesis. We found Mo17 to enhance the mutant phenotype of *Oy1* when crossed with *Oy1*/+::B73. This prompted us to use the IBM and Syn10 RIL populations to genetically dissect this differential effect of Mo17 and B73 on *Oy1*. Testcross populations of these RILs were generated by crossing with *Oy1*/+::B73, followed by evaluation of both the mutant and wild-type siblings in each progeny for leaf greenness (SPAD reading), plant height, ear height, and stem width. All of these traits showed high correlation in mutants. A single QTL, modifier of *Oy1* (*moy1*) was identified that mapped to the short arm of chromosome 10 for all traits. The *moy1* locus was validated and resolved to a ~2 Mbp region using the B73/Mo17 NIL population. In an attempt to get a better resolution of *moy1*, and also to map additional *moy* loci, a MAGIC-based GWAS was conducted by evaluating the test cross progeny of 344 maize diversity lines with *Oy1*/+::B73. A single statistically significant peak with a SNP in the middle of the *moy1* locus was found to associate with leaf greenness. Next, genotyping of three Syn10 RILs that showed recombination in the vicinity of the SNP marker identified above by GWAS narrowed down *moy1* to an interval of ~150 kbp. Cloning of the genetic variant underlying *moy1* is underway.

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P114

Fine mapping the male sterile mutant gene in maize obtained by space flight

(submitted by Yonghui Zhu <yhzhu288@gmail.com>)
Full Author List: Zhu, Yonghui[1]; Shi, Ziwen[1]; Yi, Hongyang[1]; Cao, Moju[1]
[1] Maize Research Institute of Sichuan Agricultural University, Chengdu, Sichuan 611130, China

Male sterility in flowering plant is a common phenomenon caused by abnormal anther or pollen development, while the female fertility is not influenced. A maize male sterile mutant was obtained from the offspring of maize seeds of a commercial hybrid Chuandan No.9 which had been carried into space in 1996 by satellite, named *ms39*. The *ms39* mutant is noteworthy not only for the completely and stable male sterility but also for its pleiotropic phenotypes, such as dwarf in male sterile plants. Furthermore, our previous study revealed the *ms39* was controlled by a recessive nuclear gene. Primary mapping showed *ms39* located on chromosome 3 long arm. In order to fine mapping the *ms39*, on one hand several large mapping populations including (*ms39*×Mo17) F_2, (*ms39*×B73) F_2 and (*ms39*×Mo17) BC_1F_2 were constructed, on the other hand new molecular markers were constantly designed according to the renewed mapping region. 1073 *ms39* mutant individuals derived from the (*ms39*×Mo17) F_2 population were used for fine mapping. 195 plants derived from (*ms39*×B73) F_2 population and 271 *ms39* mutant plants from (*ms39*×Mo17) BC_1F_2 population were used for verifying the mapping results.

Finally, *ms39* was mapped with in a 362kb region on chromosome 3 flanked by markers L8 (InDel) and M30 (SSR) with genetic distance 0.1cM and 0.4 cM respectively. There are 10 candidate genes within the candidate region. A segment in rice chromosome 1 is identified syntenic to our candidate region in which 6 annotated genes are all consistent with that in maize. In summary, our results lay the strong foundation for cloning the *ms39* gene and exploring molecular mechanism of anther development in maize.

P115

Functional characterization of a maize ABA hydroxylase (*Abh4*) and its contribution to carbon isotope discrimination

(submitted by Viktoriya Avramova <viktoriya.avramova@tum.de>)
Full Author List: Avramova, Viktoriya[1]; Matthes, Michaela[1]; Niculaes, Claudiu[1]; Yang, Zhenyu[2]; Rozhon, Wilfried[3]; Hoffmann, Thomas[4]; Frey, Monika[1]; Bauer, Eva[1]; Schön, Chris-Carolin[1]
[1] Plant Breeding, TUM School of Life Sciences Weihenstephan, Technical University of Munich, D-85354 Freising, Germany
[2] Botany, TUM School of Life Sciences Weihenstephan, Technical University of Munich, D-85354 Freising, Germany
[3] Biotechnology of Horticultural Crops, TUM School of Life Sciences Weihenstephan, Technical University of Munich, D-85354 Freising, Germany
[4] Biotechnology Natural Products, TUM School of Life Sciences Weihenstephan, Technical University of Munich, D-85354 Freising, Germany

During photosynthesis plants discriminate against the stable isotope ^{13}C. In plant breeding, this discrimination is used as a desirable physiological trait associated with water use efficiency (WUE) and drought tolerance. In C_3 plants, carbon isotope discrimination ($\Delta^{13}C$) is correlated with stomatal conductance. It has been shown that the homeostasis of the phytohormone abscisic acid (ABA) is a main driver of stomatal closure, therefore a link between $\Delta^{13}C$ and ABA levels can be assumed. Knowledge about these mechanisms in the C_4 species maize is limited compared to C_3 plants, because of the different nature of carbon fixation, and the fact that the metabolic pathways of ABA (which genes and proteins are involved) are not well characterized.

Using a maize introgression library, we identified lines that showed altered $\Delta^{13}C$ compared to the recurrent parent. In one of the introgressed segments an ABA hydroxylase (*Abh4*), hypothesized to be involved in the degradation of ABA, is located. Here, we functionally characterize *Abh4* through physiological and molecular approaches. We show that higher *Abh4* expression correlates with reduced ABA content and increased stomatal conductance due to both, altered stomatal aperture and development. The respective introgression line also shows increased leaf transpiration rate and decreased $\Delta^{13}C$ and WUE.

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P116

Functional characterization of maize *Phosphorus-Starvation Tolerance 1* (*ZmPstol8.02*) promoter

(submitted by Sylvia Morais de Sousa <sylvia.sousa@embrapa.br>)
Full Author List: Negri, Barbara F[1]; Ferreira, Nataly F[2]; Palhares, Patrícia LS[2]; Lana, Ubiraci GP[2 3]; Alves, Meire C[3]; Guimarães, Claudia T[1 3]; Carneiro, Andrea A[3]; de Sousa, Sylvia M[1 2 3]
[1] Universidade Federal de São João del-Rei – UFSJ, São João del-Rei, MG, Brazil, 36307-352
[2] Centro Universitário de Sete Lagoas - UNIFEMM, Sete Lagoas, MG, Brazil, 35701-242
[3] Embrapa Maize and Sorghum, Sete Lagoas, MG, Brazil, 35701-970

Phosphorus (P) is an essential macronutrient required for a range of key biochemical processes associated with plant growth and function; however, most soils throughout the world are deficient in readily available forms of P, and poor availability of P limit cereal production. Modifications in root morphology are important strategies to maximize soil exploitation under P starvation in plants. Our group performed a multiple interval QTL mapping in a maize recombinant inbred line population derived from a bi-parental cross of lines L3 and L22, P-efficient and inefficient, respectively, under low-P condition. The QTL mapping revealed candidate genes as maize homologs to *Phosphorus-Starvation Tolerance 1 (Pstol1)* that is a gene responsible to enhance root surface, P acquisition and grain yield in rice under P deficiency. One of the candidates is the *ZmPstol8.02* that co-localizes root length, root surface area, root:shoot ration and P content and was highly expressed in roots of L22, the donor line of the favorable QTL alleles. In the present study, we aimed to characterize *ZmPstol8.02* promoter region. The upstream region (-1 to -2039 bp) was analyzed using SIGNALSCAN program provided by NEW PLACE database in order to identify their cis-regulatory elements (CREs). Using this approach, we found 450 and 444 CREs in the promoter of L3 and L22, respectively. Five CREs were found in a larger number in L3. All elements that were found in a higher number in L3 are related to abscisic acid (ABA) that regulates many aspects of plant growth and development, including inhibition of root elongation. In other to validate the promoter region and better comprehend its regulation; we cloned around 2 Kb of L3 and L22 promoter region in pTF102 using *Bar* gene as a selective marker and *Gus* as a reporter. Maize HiII plants were genetically transformed via *Agrobacterium tumefaciens* EHA101 strain and regenerated from selected callus in shooting and rooting medium. Fragments of the *Bar* gene (~400 bp) and *Gus* gene (~700 bp) were amplified by PCR, confirming integration of the cassettes in the transformed plants. All events presented from one to three copies of *Bar* gene. Both promoters presented *Gus* expression levels in roots and shoots with a similar intensity as CaMV 35S promoter.

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P117

Functional studies of divergent prolamins

(submitted by Zhiyong Zhang <zhiyong@waksman.rutgers.edu>)
Full Author List: zhang, zhiyong[1]; zhang, wei[1]; Messing, Joachim[1]
[1] Waksman Institute of Microbiology, Rutgers University, 190 Frelinghuysen Road, Piscataway, NJ, US 08854-8020

Prolamins, the major storage proteins present in most cereal grains, are encoded by a large gene family that arose from a tandem duplication of an α-globulin gene. Although carbohydrates are the major calorie source in cereal grains, representing about 70% of seed weight, the proteins with only about 10% play an important part in the nutritional quality of cereals. Cereal proteins also determine important physical properties of crop seeds. For instance, the gamma prolamins in maize, also called gamma zeins, contribute to kernel hardness, which is a critical property for storage and transport of corn. On the other hand, the wheat prolamins, also known as glutens, play key roles in the rheological properties of dough, which are essential for bread and noodle products. These divergent physical properties of prolamins are reflected in the evolution of the prolamin gene family. Interestingly, despite the divergence of prolamins into different classes and chromosomal locations their regulation of gene expression appears to be quite conserved based on gene transfer from one species to another. To further characterize these physical properties, we use different evolutionary distances to express the combination of different prolamins in maize. An interesting intermediate evolutionary distance between wheat and maize is teff (Eragrostis tef), a cereal consumed mainly in Ethiopia. It is used to make a kind of flatbread called injera. While teff has in this respect similar properties as wheat, its prolamins are more closely related to the maize prolamins. For instance, it has alpha zeins instead of gliadins. However, in contrast to maize it has a higher expression and amplification of globulin genes. To test the role of globulin gene expression in endosperm, we constructed chimeric teff globulin genes with a strong alpha zein promoter for expression in maize.

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P118

Gene expression response to past, modern, and future climate conditions in maize and teosinte

(submitted by Sarah Pedersen <smpeders@iastate.edu>)
Full Author List: Pedersen, Sarah[1]; Ross-Ibarra, Jeffrey[2]; Piperno, Dolores[3]; Hufford, Matthew[1]
[1] Department of Ecology, Evolution, and Organismal Biology, Iowa State University, Ames, IA 50011, USA
[2] Department of Plant Sciences, Center for Population Biology and Genome Center, University of California, Davis, California 95616, USA
[3] Department of Anthropology, Program in Human Ecology and Archaeobiology, Smithsonian National Museum of Natural History, Washington, DC, USA.

Since the identification of teosinte as the ancestor of maize, understanding the genetic basis of domestication of maize has been of great interest. A primary focus has been the search for genes underlying the morphological differences between the two taxa. While several domestication genes have been identified, such as tb1 and tga1, there is still much to be discovered about the process of maize domestication. For example, even though it is well known that climate at the time of domestication was much different from today, many domestication studies ignore the potential impacts of environment. Previous work has shown that teosinte can undergo phenotypic plasticity when exposed to lower atmospheric carbon dioxide concentrations and temperatures similar to those found at the time of maize domestication. These plastic responses result in maize-like traits such as reduced branching and lateral branches ending in female inflorescences. The maize-like phenotypes induced by this plastic response suggest ancient teosinte may have differed dramatically from modern plants. In addition, maize shows a lack of phenotypic plasticity under past climate conditions, indicating a loss of phenotypic plasticity for branching and inflorescence traits during domestication. Based on the responses observed under past climate conditions, there is potential for future climate conditions to also induce a plastic response. To better understand the gene expression changes occurring between past and modern climate conditions and to investigate how both maize and teosinte will respond to projected future climate conditions, growth chambers were used to subject maize and teosinte to past, modern, and future climate conditions. RNA-seq was conducted on leaf tissues to determine gene expression differences between past, modern, and future climate conditions. With the use of differential expression and co-expression analyses, insights have been found regarding genes involved in response to changing climate conditions in maize.

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P119

Genome-wide expression profiling of Maize in response to polyphagous herbivore Spodoptera litura

(submitted by Indrakant Kumar Singh <iksingh@db.du.ac.in>)
Full Author List: Singh, Indrakant Kumar[1 2]; Singh, Archana[3]; Singh, Sujata[1]; Kumar, Sumit[1]
[1] Molecular Biology Research Lab., Department of Zoology, Deshbandhu College, University of Delhi, Kalkaji, Delhi-110019
[2] Department of Entomology, University of Kentucky, Lexington, KY- 40546 USA
[3] Department of Botany, Hans Raj College, University of Delhi, Delhi-110007, INDIA

Spodoptera litura (Fab.) (Lepidoptera: Noctuidae), a polyphagous insect pest, causes significant damage to crop plants throughout the world. In order to decipher the plant resistance mechanism against this voracious herbivore, we compared its growth and development on host and non-host plants and found that even though insects are able to grow and develop on a variety of host plant species; their performance was impaired on Maize. It is well known that large-scale transcriptional changes accompany insect-induced resistance, and herbivore-specific cues orchestrate the responses. Therefore, to investigate the molecular mechanism behind maize's resistance against S. litura, we performed genome wide transcript profiling of Maize genes by means of microarray approach using the custom-designed Agilent - 016047 maize 44 K microarray and we found that 3663 genes altered their expression levels during early phase of S. litura-infestation that represents approximately 9% of the total transcripts. The up-regulated genes were identified and categorized into functional classes: defense-related, oxidative stress-related, regulatory genes, protein synthesis genes phytohormone-related, primary and secondary metabolism-related. The findings of this study will provide novel insights to the molecular control of maize defense mechanism against S. litura and will be helpful in developing new strategies for crop protection that will be the most effective and economic option for integrated pest management.

P120

Genome-wide identification and classification of basic Helix-Loop-Helix (bHLH) in *Setaria spp.* and functional characterization of vascular related transcription factors through CRISPR/Cas9 mutagenesis

(submitted by Marcio Alves-Ferreira <alvesfer@uol.com.br>)
Full Author List: Lambret-Frotte, Julia[1 2 3]; Brutnell, Thomas P.[2]; Langdale, Jane[3]; Alves-Ferreira, Marcio[1]
[1] Universidade Federal do Rio de Janeiro, Cidade Universitária, Rio de Janeiro, RJ, 21941-599, Brazil
[2] Donald Danforth Plant Science Center, St. Louis, MO 63132, USA
[3] University of Oxford, Department of Plant Sciences, South Parks Road, Oxford, OX13RB, UK

The bHLH proteins form the largest family of transcription factors in plants, and regulate a diverse range of biological processes. Recently, many bHLHs have been implicated in the determination of the C4 Kranz anatomy. The aim of this study is to identify and classify the bHLH transcription factors in the *Setaria spp.* and to functionally characterize two bHLHs reported to control vascular development, an important trait in Kranz development – LHW and TMO5. The genome-wide identification of bHLHs identified 197 sequences for each *Setaria spp.* Bayesian inference successfully recovered the evolutionary history of this gene family, and from the other species analysed, *A. thaliana, O. sativa, B. distachyon, S. bicolor* and *Z. mays*. The *Setaria* species displayed great similarity, not only at the sequence level but also in genome synteny, suggesting that domestication did not affect the bHLH structure in the *S. italica* and *S. viridis* genomes. Finally, several independent CRISPR/Cas9 lines were obtained through *S. viridis* transformation. From those, six lines had a mutation in LHW-like2 with mendelian segregation; and other two lines in TMO-like1 and TMO-like3, but no homozygous plants were retrieved, suggesting lethality. Morphological analysis is ongoing to elucidate the role of these transcriptional factors in Kranz development.

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P121

HC-toxin causes massive transcriptional and metabolic changes in maize during *Cochliobolus carbonum* race 1 infection

(submitted by Kevin Chu <chu16@purdue.edu>)
Full Author List: Chu, Kevin[1]; Best, Norman B[2]; DeLeon, Alyssa[1]; Rhodes, David[2]; Dilkes, Brian P[3]; Johal, Gurmukh S[1]
[1] Department of Botany and Plant Pathology; Purdue University; West Lafayette, IN, 47907
[2] Department of Horticulture and Landscape Architecture; Purdue University; West Lafayette, IN, 47907
[3] Department of Biochemistry; Purdue University; West Lafayette, IN, 47907

The maize pathogen *Cochliobolus carbonum* race 1 (CCR1) utilizes the cyclic tetrapeptide HC-toxin as a key determinant of virulence, with strains of CCR1 unable to produce HC-toxin not able to spread beyond the initial point of infection unless HC-toxin is provided exogenously. Despite its name, HC-toxin is not cytotoxic but has been demonstrated to inhibit a broad spectrum of histone deacetylases. Previous experiments have suggested that HC-toxin is somehow shutting down defense gene expression during infection. To further clarify the role HC-toxin is playing in pathogenesis, we performed a RNA-seq study to observe and compare changes in the transcriptomes of Tox- and Tox+ CCR1-inoculated maize leaf tissue. We show here that HC-toxin actually leads to the increased expression of most defense genes during infection. Examination of the biosynthesis and signaling pathways of various defense hormones revealed increased upregulation in the Tox+ CCR1 interaction, suggesting that HC-toxin does not repress immune pathways. Amino acid extraction and quantification revealed large-scale metabolic perturbations indicative of increased protein turnover and cellular stress. We also showed the deregulation of multiple primary and secondary metabolic pathways, notably the consistent upregulation of the shikimate pathway and downregulation of the light reactions of photosynthesis by the Tox+ CCR1 interaction. These findings suggest that HC-toxin may be inducing susceptibility by inhibiting photosynthesis and deregulating metabolism, thus rapidly inducing a starvation response similar to that induced by darkness-induced loss of immunity.

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P122

Identification and characterization of maize LINC complex components

(submitted by Hardeep Gumber <hardeep@bio.fsu.edu>)
Full Author List: Gumber, Hardeep K[1]; Estrada, Amado L[1]; Bass, Hank W[1]
[1] Department of Biological Science, Florida State University, Tallahassee, FL, USA 32306-4295

The nuclear envelope is a double-membraned structure physically separating the nucleoplasm from the cytoplasm. The Linker of Nucleoplasm to Cytoplasm (LINC) complexes act as bridges across this envelope. LINC complexes maintain the nuclear shape and architecture, regulate nuclear and chromosome movement during cell division, organize chromatin, and regulate transcription of stress responsive genes. The LINC complex tethers moving telomeres to the nuclear envelope during meiotic prophase, when homologous chromosomes synapsis and recombination occur, as needed for chromosome segregation and transmission. The LINC complex is comprised of proteins from four major cellular areas: (1) Lamins or CRWNs at the chromatin periphery in the nucleus, (2) SUN domain proteins in the INM of the NE, (3) KASH domain proteins in the ONM of the NE, and (4) cytoskeleton and other proteins in the cytoplasm around the nuclear periphery. LINC complexes for plants are generally not yet defined, with components having a wide range of conservation, from highly conserved (SUN, tubulin, actin) to fast evolving (KASH and nuclear proteins). The maize KASH gene family was estimated to be encoded by at least 14 different gene loci. Some maize KASH candidate genes have Arabidopsis homologs, while others appear limited to monocots or maize. To define the maize LINC complex, we have taken informatic and biochemical approaches, leading to the detection of two CRWNs (crowded nuclei), lamin-like nuclear filamentous proteins, four KASH candidates, and multiple cytoskeletal and motor proteins. KASH validation experiments involve screening candidates using RFP and affinity tagged fusion protein constructs. Additional genetic analyses using Mu-tagged alleles and colchicine disruption are underway to test the prediction that SUN knockout plants will show perturbed or failed meiotic prophase. These multiple and complementary approaches are defining the structure and function of the maize LINC complexes, while revealing new candidates for cellular coordination of nuclear processes.

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P123

Identification of phospholipid metabolic patterns involved in maize adaptation to highland conditions

(submitted by Rubén Rellán-Álvarez <ruben.rellan@cinvestav.mx>)
Full Author List: Juárez-Núñez, Karla[1]; Fiehn, Oliver[2]; Sawers, Ruairidh[1]; Rellán-Alvarez, Rubén[1]
[1] National Laboratory of Genomics for Biodiversity, Cinvestav, Irapuato, Guanajuato, Mexico.
[2] West Coast Metabolomics Center, UC Davis, Davis, California, USA.

Phospholipids are major building blocks of cell membranes and important signaling compounds. Under low phosphorus, plants reduce the concentration of phospholipids to free phosphate and increase the abundance of galactolipids and sulpholipids. Conversely, at low temperatures, plants increase the concentration of phospholipids in membranes to maintain membrane fluidity. After domestication from lowland teosinte (*Zea mays ssp. parviglumis*), maize colonized the Trans-Mexican volcanic belt highlands that are characterized lower temperatures and phosphorus bioavailability of phosphorus. Similar environmental conditions were encountered in South-American highlands in a second independent event of maize highland adaptation.
We hypothesize that low temperature and phosphorus bioavailability were major selective forces during maize adaptation to the highlands and shaped phospholipid metabolism. Recent data has shown that genes with high differentiation between highland and lowland sites are related with phospholipid metabolism.To identify the genetic architecture of phospholipid metabolism and its role on maize highland adaptation we grew a maize RIL mapping population (developed using a Mexican highland landrace Palomero Toluqueño and B73 in common garden experiments in Mexico at sea level and at 2600 masl and sampled leaves at V4 stage. Using UPLC-QTOFMS we identified around 30 phospholipid species that were used as phenotypes for QTL analysis. We identified a QTL peak at 86Mb in chr3 for phosphatidylcholine (PC) and lyso-phosphatidylcholine (lyso-PC). A candidate gene at the QTL peak has a putative PC to lyso-PC enzymatic activity and is upregulated in cold conditions. Further analysis using a landrace diversity panel suggest that this biochemical phenotype might have been selected for in highland Mexican maize, but not in South-American highland lines.

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P124

Identifying the genetic mutation responsible for *carbohydrate partitioning defective 28/47*

(submitted by Kyle Conner <krcp7c@mail.missouri.edu>)
Full Author List: Conner, Kyle[2]; Julius, Ben[2]; Keefe, Peter J.[1]; Bihmidine, Saadia[2]; Baker, R. Frank[2]; Chomet, Paul[3]; Wagner, Ruth[3]; Grote, Karen[3]; Peevers, Jeanette[3]; Lubkowitz, Mark[1]; Braun, David M.[2]
[1] Department of Biology, Saint Michael's College, Colchester, VT 05439, USA
[2] Division of Biological Sciences, Interdisciplinary Plant Group and the Missouri Maize Center, University of Missouri, Columbia, MO 65211, USA
[3] Monsanto, Chesterfield, MO 63017 USA

The process of converting inorganic carbon dioxide into carbohydrates predominantly occurs in photosynthetic leaves (source tissue). These carbohydrates are allocated to various non-photosynthetic sink tissues (e.g., roots, stems, flowers, and fruits) for proper growth and development, in a process termed carbohydrate partitioning. While much is known about the physiology of carbon transport, we have little knowledge of the genetics that govern it. Therefore, we are taking a genetic approach to identify mutants unable to properly transport sugars, which we call *carbohydrate partitioning defective* (*cpd*). To identify *cpd* mutants, we screened for plants displaying visible phenotypes suggestive of inhibited sugar transport: leaf chlorosis, starch accumulation in leaves, and decreased plant growth. Two mutants displaying these phenotypes, *cpd28* and *cpd47*, were found to allelic through complementation testing. In addition to the visible phenotypes, *cpd47* exhibits increased lignin deposition in the phloem of major and minor veins, and decreased leaf chlorophyll levels, stomatal conductance, and photosynthetic rates. Through bulk segregant analysis (BSA), we determined that the mutations responsible for the *cpd28/47* mutants were located on the long arm of chromosome 1. Genetic fine-mapping enabled us to narrow this region. To pinpoint the causative gene, whole genome sequencing was performed on pools of *cpd28* and *cpd47* mutants, and an analysis of the SNPs between each mutant and the B73 reference genome resulted in the identification of a single gene. Further research and understanding of the gene's function will help provide knowledge that will aid in future advancements in increasing crop yield and growth.

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P125

Increased lysine and methionine accumulation in single maize seeds by different mechanisms

(submitted by Jose Planta <joplanta@scarletmail.rutgers.edu>)
Full Author List: Planta, Jose[1]; Messing, Joachim[1]
[1] Waksman Institute of Microbiology, Rutgers, The State University of New Jersey, 190 Frelinghuysen Road, Piscataway, New Jersey 08854-8020

The essential amino acids lysine and methionine are limiting factors in a maize-based diet. In animal feed, corn meal is supplemented with soybeans to compensate lysine and chemically synthesized methionine because both are deficient in methionine. To explore a sole crop diet, we investigated whether an approach can be taken to correct for both deficiencies in maize. Previously, it was shown that knockdown of the abundant α-zeins increases seed lysine content. In a separate line of investigation, we created a transgenic event in maize, PE5, where reduced sulfur was increased in leaves because the PE5 event expresses the *PEPC* promoter-driven *E. coli* enzyme 3'-phosphoadenosine-5'-phosphosulfate reductase, an enzyme involved in reductive sulfate assimilation. As a result, the sulfur sink exhibits an increased seed methionine content when used as the maternal parent. Now, we crossed PE5 to RNAi lines targeting the α-, β-, and γ-zeins. These crosses were made to: (i) determine the effects of a high-methionine parent to lysine accumulation in different zein knockdown backgrounds and (ii) if both methionine and lysine can be mutually increased using these parental combinations. Of the three RNAi lines and their combinations, only PE5/αRNAi+ and PE5/αRNAi+/γRNAi+ kernels showed statistically different lysine content from their respective controls of non-RNAi segregating kernels from the same ears, leading to a 60.0% and 128.2% increase in lysine, respectively. PE5/αRNAi+ kernels have 7.25 mol% of lysine while PE5/αRNAi+/γRNAi+ kernels have 5.29 mol%; this difference in lysine content from their respective controls and the actual values suggests that the hybrid genetic backgrounds of these two groups have an impact on lysine accumulation. The single-transgene αRNAi line has a 26.7% increase in lysine while PE5/αRNAi+ kernels have 151.7% more lysine compared to non-transgenic controls, suggesting that the high-methionine PE5 maternal background also influences lysine accumulation.

P126

Independent of arbuscular mycorrhizal symbiosis: A novel arbuscular mycorrhizal mutant in maize

(submitted by Bethan Manley <bm502@cam.ac.uk>)
Full Author List: Manley, Bethan[1]; Riahy, Darius[1]; Li, Bailin[2]; Paszkowski, Uta[1]
[1] Department of Plant Sciences, University of Cambridge, Cambridge CB2 3EA, United Kingdom
[2] DuPont Pioneer, Johnston, Iowa 50131

Arbuscular mycorrhizal fungi from the *Glomeromycota* phylum form a beneficial symbiotic relationship with the root systems of a majority of plant species, enabling plants to profit from increased phosphate uptake efficiency. Further understanding of this interaction may prove vital in meeting the challenge of an increasing demand for phosphate supply in agriculture. Establishment of this symbiotic interaction relies on molecular crosstalk between the fungus and the plant roots, and requires rearrangement of the architecture of fungal-accommodating root cortical cells. Forward genetic screens have enabled identification of the genetic components underlying these processes. The *independent of arbuscular mycorrhizal symbiosis* (*ina*) mutant is perturbed at early stages of the interaction with the arbuscular mycorrhizal fungus, as reflected by the severely compromised root colonisation phenotype. This project will utilise positional cloning methods to identify and characterise the causal mutation of the *Zea mays* mutant *ina*.

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P127

Inducible defense metabolites in maize as a platform for the discovery of antibiotics, mode of action, and biosynthetic pathways

(submitted by Andrew Sher <awsher@ucsd.edu>)
Full Author List: Sher, Andrew[1]; Liu, Roland[2]; Pogliano, Kit[2]; Huffaker, Alisa[1]
[1] Section of Cell and Developmental Biology, University of California, San Diego, La Jolla, CA
[2] Section of Molecular Biology, University of California, San Diego, La Jolla, CA

In response to attacking organisms, maize produces a rich and diverse suite of specialized defense metabolites that include, but are not limited to benzoxazinoids, phenylpropanoids, terpenoids and oxylipins. We seek to explore, identify and ultimately exploit maize specialized metabolites through bioprospecting for novel candidate antimicrobials. Research into new antimicrobial compound leads are urgently required due to the rapidly growing number of microbes resistant to current antibiotics. While most antibiotics are derived from microorganisms, plants remain an underutilized resource for the discovery of bioactive chemicals useful either agriculturally or pharmaceutically. To search for candidate antimicrobial agents, we generated tissues strongly enriched in defense metabolites by eliciting diverse field-grown inbred lines with heat-inactivated Fusarium hyphae. Pooled elicited maize tissues were extracted with methanol and fractionated using high-resolution reverse phase C18 flash chromatography. Individual C18-derived fractions were tested for antibiotic activity against E. coli in microscale quantities using differential staining of cells and microscopy to identify potential mechanisms and categories of bactericidal or bacteriostatic activities. Subsequent fractionation using semi-preparative C18 high performance liquid chromatography (HPLC) coupled with antibiotic bioassays revealed numerous individual separable activities that inhibited bacterial growth through distinct mechanisms. Analyses of these fractions by mass spectrometry revealed a novel diterpenoid, and additional candidates of diverse biosynthetic origins which are being purified for identification and activity assays. In parallel with the identification of maize-derived antibiotics, metabolite-based Genome Wide Association Studies (GWAS) are being conducted to identify regulatory loci associated with biosynthesis. Bioassay-driven approaches enable the discovery and research prioritization of both biochemical and genetic efforts (forward and reverse) required to uncover pathways useful for protecting both maize and potentially man.

P128

Investigating the roles of jasmonic acid and cytokinin in maize leaf growth control

(submitted by Aimee Uyehara <anu42@hawaii.edu>)
Full Author List: Uyehara, Aimee N.[1]; Cahill, James[1]; Hunter, Charles[2]; Muszynski, Michael G.[1]
[1] Dept. of Tropical Plant and Soil Sciences, University of Hawai'i at Mānoa, Honolulu, HI
[2] Chemistry Research Unit, CMAVE-USDA, Gainesville, FL

Plant growth is the accumulation of biomass attributed to cell division and cell expansion. In the maize leaf, growth is spatially separated into three distinct growth zones: the division zone, elongation zone, and the maturation zone. This spatial separation makes the maize leaf a useful model for understanding growth because changes in the cellular morphology of these growth zones reflects changes in the underlying molecular networks driving growth. We previously showed that the semi-dominant maize mutant, *Hairy Sheath Frayed (Hsf1)* was caused by hypersignaling of the plant hormone cytokinin (CK) which reduced leaf size. CK typically promotes cell division but can repress growth in certain tissues. Analysis of *Hsf1* leaves indicated there was a decrease in the number of dividing cells which was associated with increased levels of jasmonic acid (JA), a defense and growth repressive hormone. JA is known to reduce cell division in *A. thaliana* implying that CK-signaling may restrict maize growth through upregulating the JA pathway. To test this idea, we adapted our maize seed hormone assay to establish the effects of exogenous JA application on maize leaf growth. B73 seeds treated with JA exhibited a significant reduction in leaf size and a decreased leaf elongation rate. Next, JA treatments were done on *Hsf1/+* and WT-sib seedlings. JA treatments resulted in a greater reduction in leaf size in WT-sibs compared to JA-treated *Hsf1/+*, suggesting that JA perception is already saturated in *Hsf1* mutants. Treatments are being repeated to confirm these results. These data help explain the cause of growth reduction in *Hsf1* and set the foundation for further molecular and histological tests of JA-mediated growth changes downstream of CK signaling.

P129

Large-scale molecular characterization of integration sites and T-DNA structures in *Agrobacterium*-mediated transgenic events of maize inbred B104

(submitted by Anjanasree Neelakandan <anjanakn@iastate.edu>)
Full Author List: Neelakandan, Anjanasree K.[1]; Kabahuma, Mercy[1 2]; Lopez, Miriam[1 3]; Yang, Qin[4]; Balint-Kurti, Peter[4 5]; Lauter, Nick[1 2 3]
[1] Department of Plant Pathology and Microbiology, Iowa State University, Ames, Iowa, 50011
[2] Interdisciplinary Genetics and Genomics Graduate Program, Iowa State University, Ames, Iowa, 50011
[3] USDA-ARS, Corn Insects and Crop Genetics Research Unit, Ames, Iowa, 50011
[4] Department of Plant Pathology, North Carolina State University, Raleigh, North Carolina, 27695
[5] USDA-ARS, Plant Science Research Unit, Raleigh, North Carolina, 27695

Agrobacterium-mediated genetic transformation has been widely used for functional studies and for developing superior traits in maize as well as other crops. Maize inbred B104 is amenable to *Agrobacterium*-mediated transformation and its genome sequencing is in progress, making B104 transformants a useful model for dissecting the features of T-DNA integration in maize. Determining transgene integration sites permits analysis of the genetic environment of each event, establishes genetic traceability of events, and can predict and/or account for event-specific outcomes, such as disruption of an endogenous gene. Additionally, analysis of junction sequences at the insertion sites is important for documenting which molecular mechanisms participate in T-DNA integration. Determining the T-DNA structure for each insertion event is also important; copy number affects expression levels and propensity for being silenced by the host genome, while T-DNA truncation and super-inclusion of plasmid backbone sequences can have functional consequences, particularly if antibiotic-resistant selectable markers are present in T-DNAs with improper borders.
In the present study, we are characterizing the B104 integration sites and T-DNA structures for ~150 independent events derived from a pMCG1005-based set of 16 different transgene constructs. Flanking sequences are being identified using a maize-customized TAIL-PCR approach that is efficient and specific. Thus far, we have been able to infer that T-DNA insertions preferentially occur in gene-rich regions, if not in genic sequences. We have also identified three types of junctions, expanding our understanding of the T-DNA integration process. T-DNA compositions are being determined using a series of diagnostic PCR assays. With these, we are identifying the subset of events that are single copy, as well as those that contain antibiotic-resistant selectable marker genes, which are of interest for regulatory compliance. These insights will be important for streamlining functional studies of these events and could be used to improve transgenesis methods.

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P130

Maize secondary metabolites inhibit carboxymethylcellulose-driven low-grade colonic inflammation and metabolic syndrome

(submitted by Shara Chopra <soc5439@psu.edu>)
Full Author List: Bhatnagar, Rohil[1]; Indukuri, Vijay Kumar[2]; Chopra, Shara[1]; March, Kylie[1]; Vanamala, Jairam[2]; Chopra, Surinder[1]; Reddivari, Lavanya[1]
[1] Department of Plant Science, The Pennsylvania State University, University Park, PA 16802, USA
[2] Department of Food Science, The Pennsylvania State University, University Park, PA 16802, USA

Diet plays a pivotal role in defining human health outcomes. Western diet characterized by high- fat, processed foods, and sugary desserts has been shown to increase the risk for inflammation and related disorders such as obesity, cancer etc. Certain components of processed foods are dietary emulsifiers, such as carboxymethylcellulose (CMC), that can cause a heightened immune response and promote inflammation, progressing to chronic disorders. Plant metabolites, especially certain secondary metabolites (SM) such as phenolics (P), have been shown to have anti-inflammatory, anti-carcinogenic and anti-atherogenic properties. Studies have shown that the beneficial properties of SMs are associated with the human gut microflora. A balance in the human gut microbiome exists between the beneficial and potentially harmful microbiota communities. In this study, we postulated that a specific class of bioactive SMs present in maize would significantly alleviate inflammation induced by CMC in C57BL/6 mice model. We also hypothesized that this alleviation of inflammation could be because of the restoration of intestinal mucosal barrier function. In this study, we thus characterized the consequences of CMC on the colonic health of C57BL/6 mice. To accomplish the goals of this research, mice were fed specific diets containing corn meals from genotypes with and without selected SMs along with CMC and controls. CMC treatment resulted in increased weight gain, adiposity, fasting glucose levels and reduced colon length. Supplementation with corn meal containing selected SMs alleviated the deleterious effects of CMC by improving the mucus thickness and reducing the colonic permeability. Clinical intervention studies need to be carried out to accurately determine the extent to which maize SM intake can improve human health.

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P131

Maize terpene synthases 6 and 11 are required for zealexin production and protection against multiple pathogens

(submitted by Philipp Weckwerth <pweckwerth@ucsd.edu>)
Full Author List: Weckwerth, Philipp R[1]; Yang, Bing[2]; Schmelz, Eric[1]; Huffaker, Alisa[1]
[1] Section of Cell and Developmental Biology, University of California San Diego, La Jolla, CA 92093
[2] Depart. of Genetics, Development and Cell Biology, Iowa State University, Ames, Iowa 50011

Acidic terpenoid phytoalexins, zealexins and kauralexins, are dominant inducible antimicrobial metabolites in maize (*Zea mays*) following the combined pressures of herbivory and microbial infection. Zealexins were first isolated and identified through efforts to characterize stalk rot (*Fusarium graminearum*)-induced defenses. Zealexins are predicted to be synthesized *de novo* by terpene synthases from farnesyl diphosphate precursors into the rarely encountered β-macrocarpene olefin and modified by a series of oxidative reactions mediated by cytochrome P450 enzymes. Zealexin accumulation is associated with increased expression of the terpene synthases *Tps6* and *Tps11*, which have been demonstrated *in vitro* to catalyze production of β-macrocarpene.
Despite proposed relationships, it remains to be empirically demonstrated that the endogenous biosynthesis of zealexins requires the exclusive activity of Tps6/11. Moreover, while select zealexins demonstrate significant *in vitro* antimicrobial activity, a combination of genetic and biochemical evidence causally linking zealexins to pathogen resistance *in vivo* is required. Using CRISPR we created *tps6* and *tps11* double mutants with frame shift mutations yielding non-functional copies of both tandemly-arrayed genes. Recent analyses utilizing liquid chromatography mass spectrometry (LC/MS) established that maize *tps6/tps11* double mutants plants lack pathogen-induced zealexins. In contrast, synthesis of diterpenoid defenses, such as the *ent*-15-kaurene-derived kauralexins, remained unchanged. Plant-pathogen bioassays revealed impaired disease resistance of *tps6/11* double mutants compared to wild type Tps6/11 plants, as measured through increased symptoms and colonization following necrotrophic fungi (*Fusarium graminearum*) and also xylem-dwelling bacteria (*Pantoea stewartii*) challenge.
Twelve years ago, Tps6/11 were demonstrated to be among the most strongly elicited transcripts in maize following pathogen attack and remain dominant inducible markers in a majority of studies. We now provide conclusive genetic, biochemical and physiological evidence that maize zealexin production requires Tps6/11 and mediates broad-spectrum defense against both fungal and bacterial pathogens.

P132

Mapping and characterizing the *Carbohydrate partitioning defective29* mutant

(submitted by Nathaniel Boyer <nrb2bd@mail.missouri.edu>)
Full Author List: Boyer, Nathaniel R.[1]; Tran, Thu M.[1]; Bihmidine, Saadia[1]; Braun, David M.[1]
[1] Division of Biological Sciences, Interdisciplinary Plant Group and the Missouri Maize Center, University of Missouri, Columbia, MO 65211, USA

Carbohydrate partitioning is the process of distributing carbon that is assimilated into sucrose in source tissues (e.g., leaves) to the non-photosynthetic sink tissues (e.g., seeds, roots, stems). Plants with mutations in the genes controlling sugar metabolism and allocation are unable to effectively transport sucrose throughout the plant, and are therefore deemed carbohydrate partitioning defective (cpd). The aim of this study is to characterize *Cpd29*, a dominant mutant. *Cpd29* mutant plants hyperaccumulate starch and soluble sugars in the leaves. In association with this overabundance of carbohydrates, *Cpd29* mutant plants showed a number of notable phenotypes, such as the accumulation of anthocyanin in leaves, chlorosis of the leaves, stunted plant growth, and reduced rates of photosynthesis and gas exchange. The gene responsible for the *Cpd29* mutation was determined to be on the short arm of chromosome 10 by Bulk Segregant Analysis (BSA) mapping. Fine mapping using polymorphic PCR-based markers narrowed down the gene's location. Once the causative gene responsible for the *Cpd29* mutation is identified, we will determine the specific role it plays in carbohydrate transport and allocation in maize. With the identification and further characterization of this gene, we will expand our understanding of carbohydrate partitioning, and apply this knowledge to increase crop yield and food security.

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P133

Metabolite-based genome-wide association studies (mGWAS) enable the efficient discovery of maize genes regulating specialized metabolism

(submitted by Eric Schmelz <eschmelz@ucsd.edu>)
Full Author List: Schmelz, Eric[1]; Ding, Yezhang[1]; Weckwerth, Philipp[1]; Jander, Georg[2]; Philipp, Zerbe,[3]; Sibongile, Mafu[3]; Alisa, Huffaker[1]
[1] Section of Cell and Developmental Biology, University of California, San Diego, La Jolla, CA
[2] Boyce Thompson Institute for Plant Research, Ithaca, NY, 14850
[3] Department of Plant Biology, University of California, Davis, Davis, California, USA

Maize production in the United States spans 120,000 square miles of arable land that yields over 10 billion bushels to fuel a complex 1.7 trillion dollar market value chain. Annually planted monocultures are also large-scale resources for complex arrays of pests and pathogens capable of overcoming resistance mechanisms. Maize seedlings are largely protected from biotic agents by a complex suit of benzoxazinoid defenses that have been intensively studied genetically and biochemically for over 60 years. Modern advances in analytical chemistry now reveals that dominant inducible defenses mediating resistance are exceedingly complex and span hundreds of specialized metabolites from diverse biochemical pathways. To broadly capture regulatory loci and specific biosynthetic genes responsible, we leveraged the Goodman diversity panel of 282 inbreds and GWAS to identify metabolite QTLs (mQTLs). Underlying gene candidates are effectively narrowed further using RNA-seq based transcriptomics to confirm paired behavior. Preliminary studies resulted in highly significant mQTLs for over 100 different metabolite *m/z* features in a single tissue and time point. To validate the utility of this approach, efforts focused on completing the steps involved in diterpenoid phytoalexin biosynthesis, specifically the Kauralexin pathway. Two GWAS based mQTL gene candidates were selected for biochemical confirmation using *in vitro* enzyme assays and analyses of transposon insertion mutants to prove endogenous relationships. Additional mQTL were identified for diverse specialized metabolic pathways including benzoxazinoids, sesquiterpenoids, diterpenoids, 9-lipoxygenase derived cyclopente(a)none death acids, hydroxyfatty acids, flavonoids, hydroxycinnamic acid amides, and many unknown defense candidates. Expansion of the approach to different tissues, time points and types of elicitation events will enable the annotation of hundreds of maize genes controlling the production of specialized metabolites underlying critical biochemical resistance mechanisms.

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P134

Microarray analysis of Maize's early response to mechanical wounding and its comparison to Insect Attack

(submitted by Archana Singh <archanasingh@hrc.du.ac.in>)
Full Author List: Singh, Archana[1 2]; Singh, Indrakant Kumar[3]; Kumar, Sumit[3]; Singh, Sujata[3]
[1] Department of Botany, Hans Raj College, University of Delhi, Delhi-110007, INDIA
[2] Department of Plant Pathology, University of Kentucky, Lexington, KY-40546, USA
[3] Molecular Biology Research Lab., Department of Zoology, Deshbandhu College, University of Delhi, Kalkaji, Delhi-110019

Mechanical wounding injures plant tissues and causes cell disruption, desiccation, metabolite oxidation, disruption of primary metabolism and also provide means for pathogen invasion. In order to monitor plant responses to wounding at genomic level, we applied microarray approach using the custom-designed Agilent - 016047 maize 44 K microarray, in which 1600 transcripts were found to be up-regulated and 1359 down-regulated. Approximately 7.03% of the transcripts were altered during early phase of wounding. This analysis revealed that wounding regulates many defense-, osmotic stress-, abiotic stress-, phytohormone-related genes and modifies primary and secondary metabolic pathways. Studies of expression patterns of these genes provide new information on the interactions between wounding and other signals, including pathogen attack, abiotic stress factors, and plant hormones. Notably, insect feeding also causes mechanical wounding; therefore we compared insect attack and mechanical wounding which revealed that there are few genes that are commonly altered and significant number of transcripts that are unique to be up-regulated only during insect attack. These results further dissected the nature of mechanical wounding as a stress signal and identified new genes that may play a role in wounding and other signal transduction pathway and indicates correlation between wounding and insect attack.

P135

Miniature seed 2109 encodes a putative nitrate transporter 1/peptide transporter protein required for maize seed development

(submitted by Bo Yang <15101126267@163.com>)
Full Author List: Yang, Bo[1]; Wang, Jing[1]; Lai, Jinsheng[1]
[1] State Key Lab of Agrobiotechnology and National Maize Improvement Center Department of Plant Genetics and Breeding, China Agricultural University, Beijing 100193, China

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 especially in endosperm development from an EMS-mutagenized mutation pool of B73. Map-based cloning and allelic hybrid demonstrate that mutation in the *miniature seed 2109* (*mn2109*) mutation behaved as a monogenic recessive trait that results in maize seed developmental defects. Gene cloning and characterization indicate that *MN2109* encodes a putative transporter that belongs to the NRT1/PTR family. This conclusion is based on findings indicating that MN2109 contains a conserved PTR2 region and 12 transmembrane domains, and that the MN2109-GFP fusion protein is localized in the plasma membrane. The *MN2109* gene is specially expressed in the BETL cell type of maize endosperm, which is consistent with the predicted function of MN2109 and its phenotype. In further study, we find the BETL in *mn2109* was impaired compared with its wild type, consistent with this seed phenotype, the expression of *ZmMRP1* as the transcriptional master regulator for forming BETL cell type during seed development reduced strongly in *mn2109* seeds; this possibly causes the failure in forming BETL, thereby affects transfer of nitrogen content, sugars and other nutrients imported from the maternal phloem. Our study reporting that MN2109 may be able to function as either a nitrate or peptide transporter, is thus critical to better understanding endosperm development and seed filling in maize.

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P136

Molecular characterization and transcriptional dynamics of the jasmonate signaling pathway in maize and setaria

(submitted by Christine Shyu <CShyu@danforthcenter.org>)
Full Author List: Shyu, Christine[1]; Maxson-Stein, Kimberly[1]; Mayfield-Jones, Dustin[1]; Kumar, Indrajit[1]; Brutnell, Thomas P.[1]
[1] Donald Danforth Plant Science Center, Saint Louis, MO 62132

The plant hormone jasmonate (JA) regulates a broad range of agriculturally important traits from reproductive growth to defense against biotic and abiotic stresses. Thus, understanding the JA signaling pathway in economically important crops such as maize provides opportunities for engineering crops with enhanced JA-mediated responses. The JA receptor complex consists of an F-box protein CORINATINE INSENSITVE1 (COI1) and JASMONATE ZIM-DOMAIN (JAZ) transcriptional repressors. Upon perceiving bioactive JA, JAZ proteins are ubiquitinated and targeted for degradation. This leads to activation of JAZ-interacting transcription factors (TF) and JA-responsive gene expression. Unlike dicot systems where there is only one *COI* gene, panicoid grasses including maize, sorghum, and Setaria have multiple *COI*s (*COI1a*, *COI1b*, and *COI2*). We identified maize *coi* mutants using *Ac/Ds* and *Mutator* platforms to study the biological function of each *COI*. To fast track functional characterization of *COI*s, we utilize *Setaria viridis* as a model for genetic, cell biological and molecular genetic studies. COI-JAZ interactions were examined and CRISPR-induced mutants targeting *COI*s were generated. Three independent *coi1b* alleles were identified in *S. viridis* and preliminary phenotypic differences were observed. To identify TFs involved in JA signaling, we conducted an RNAseq experiment following wounding of developing leaf samples. Tissue was harvested from different segments across the leaf developmental gradient and at multiple time points. Nine bHLH TFs co-expressed with *JAZ*s in this dataset were identified as candidates for controlling JA responses. Functional characterization of these TFs is underway. Taken together, these studies highlight the dynamic and complex regulation of JA responses in maize and Setaria, and provide opportunities to engineer crops for enhanced stress resistance without compromising growth.

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P137

MYB31/MYB42 syntelogs exhibit divergent regulation of phenylpropanoid genes in maize, sorghum and rice

(submitted by John Gray <jgray5@utnet.utoledo.edu>)
Full Author List: Agarwal, Tina[4]; Doseff, Andrea I.[2 3]; Grotewold, Erich[1 2]; Gray, John[4]
[1] Center for Applied Plant Sciences (CAPS), The Ohio State University, Columbus, OH 43210
[2] Department of Molecular Genetics, The Ohio State University, Columbus, OH 43210
[3] Department of Physiology and Cell Biology, Heart and Lung Research Institute, The Ohio State University, Columbus, OH 43210
[4] Department of Biological Sciences, University of Toledo, Toledo, Ohio 43606 USA

ZmMYB31 and ZmMYB42 are R2R3-MYB transcription factors implicated in the regulation of phenylpropanoid genes in maize. Here, we tested the hypothesis that the regulatory function of MYB31 and MYB42 is conserved in other monocots, specifically in sorghum and rice. We demonstrate that syntelogs of MYB31 and MYB42 do bind to phenylpropanoid genes that function in all stages of the pathway and in different tissues along the developmental gradient of seedling leaves. We found that *caffeic acid O-methyltransferase (COMT1)* is a common target of MYB31 and MYB42 in the mature leaf tissues of maize, sorghum and rice, as evidenced by Chromatin immunoprecipitation (ChIP) experiments. In contrast, *4-coumarate-CoA ligase (4CL2)*, *ferulate-5-hydroxylase (F5H)*, and *caffeoyl shikimate esterase (CSE)*, were targeted by MYB31 or MYB42, but in a more species-specific fashion. Our results revealed MYB31 and MYB42 participation in auto- and cross-regulation in all three species. Apart from a limited conservation of regulatory modules, MYB31 and MYB42 syntelogs appear to have undergone subfunctionalization following gene duplication and divergence of maize, sorghum, and rice. Elucidating the different regulatory roles of these syntelogs in the context of positive transcriptional activators may help guide attempts to alter the flux of intermediates towards lignin production in biofuel grasses. These experiments have been reported recently in *Nature Scientific Reports* 2016: 6:28502, DOI: 10.1038/srep28502. This research was funded by NSF grant IOS-1125620.

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P138

Network analysis of maize kernel development in response to variable nitrogen

(submitted by Edward Ross <ehross3@illinois.edu>)
Full Author List: Ross, Edward[1]; Boddu, Jay[1]; Moose, Stephen P[1]
[1] Department of Crop Sciences, University of Illinois Urbana-Champaign, 1102 S Goodwin Ave, Urbana, IL 61801

Nitrogen (N) fertilizers are a major pollutant and input cost of maize (*Zea mays*) production, but their negative effects can be mitigated through the development of cultivars with higher nitrogen use efficiency (NUE). Yield increases due to N fertilizers are primarily attributed to increases in kernel number, a yield component that is determined early in kernel development. Responses to N at this early stage of development are difficult to investigate, due to the complex path of N within the plant and difficulties in precisely manipulating N supply at the developing kernel. To gain more control of N metabolites supplied to the developing kernel, an *in vitro* kernel culturing system was employed. Hybrid plants from crosses of B73 to Mo17, IHP1, and ILP1 were grown under variable N in the field. Developing kernels were dissected three days after pollination and placed in culture with variable N. B73 X Mo17 kernels were assayed with RNA sequencing and metabolite profiling of free amino acids. Trait and gene expression data were integrated using weighted gene correlation network analysis (WGCNA). A subset of gene modules was found to be highly correlated to free amino acid levels, either in cob or kernel tissue. GO term enrichment analysis of these modules indicates that their members are involved in carbohydrate metabolism, N metabolism, DNA packaging, and protein modification. Additionally, these modules contain genes orthologous to components of an N responsive transcriptional network identified in the *Arabidopsis thaliana* root. Alleles of these genes containing UniformMu transposon insertions have been obtained from the Maize Genetics Cooperation Stock Center, and are currently being introgressed into various backgrounds. Two of these backgrounds are the IHP1 and ILP1 inbred lines from the Illinois Long Term Selection Experiment for kernel protein concentration, which represent the extremes of N utilization efficiency in maize.

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P139

Non-destructive detection root exudate localization by blotting roots with colorimetric papers

(submitted by Truc Doan <truchuu.doan@doane.edu>)
Full Author List: Doan, Truc[1]; Doan, Tu[1]; Kangas, Michael[1]; Wilson, Christina[1]; Lukowicz, Rachel[1]; Ernest, AdreAnna[1]; Holmes, Andrea E[1]; Doyle, Erin[1]; Durham Brooks, Tessa[1]
[1] Doane University, Crete, NE, 68333

A non-destructive detection assay was developed to observe and localize the production of exudates on the root surface of maize seedlings throughout its early development. Exudates are a chemically diverse array of inorganic acids, amino acids, proteins, sugars, and other metabolites secreted by roots. Exudates provide a unique chemical signature that can vary from plant to plant, across developmental stages, and in response to stress. Greater understanding of exudate composition and localization to root structures during the plant lifespan will facilitate development of agricultural innovations that target the rhizosphere. Building off of previously developed detection methods, ninhydrin, a sensor commonly used to detect free amine groups, was inkjet printed onto commercially available tissue paper. The paper was then used to 'blot' the root to obtain an image of free amine-containing exudates (such as amino acids) on the root surface by gently pressing the paper to the root. Finally, the resulting image of the developed ninhydrin paper was overlaid with a root image to localize exudate production to root structures. The planting, blotting, and imaging components of this novel method are accessible, expanding opportunities to study exudates and their significance. Image analysis tools are currently being developed to increase the method throughput for larger studies. This novel method, once fully developed, will provide a non-destructive way for determining how genetic and environmental factors influence exudate production.

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P140

Omics analyses reveals a critical role for autophagy in reorganizing the maize metabolome, transcriptome, and proteome during nitrogen stress

(submitted by Fionn McLoughlin <fmcloughlin@wustl.edu>)
Full Author List: McLoughlin, Fionn[1]; Augustine, Robert C.[1]; Marshall, Richard M.[1]; Kirkpatrick, Liam D.[1]; Li, Faqiang[2]; Vierstra, Richard D.[1]
[1] Department of Biology, Washington University in St. Louis, St. Louis, MO, USA, 63130.
[2] Department of Genetics, University of Wisconsin, Madison, WI, USA, 53706.

Autophagy plays an important role in plant nutrient recycling by removing membranes, protein complexes, and organelles when unneeded or become dysfunctional. The pathway begins with the entrapment of cellular material into double membrane-bound autophagosomes and ends with deposition of the internal vesicles bearing cargo into the vacuole for breakdown. Accordingly, plants defective in autophagy accumulate aberrant proteins/organelles, and are sensitive to nutrient limitations. Central to the process is autophagy-related (Atg)-8, which coats the enveloping autophagosome after its conjugation to phosphatidylethanolamine (PE); the Atg8-PE adduct then provides a docking platform for factors that drive closure and vacuolar fusion, and for receptors that select appropriate cargo. This lipidation is directed by a ligase complex containing the Atg12-Atg5 adduct. To better define the cellular processes impacted by autophagy and its roles in crop yield, we combined proteomic, transcriptomic, and metabolomic approaches to study how two atg12 alleles impact the profile of RNAs, proteins, and metabolites upon nitrogen stress. Homozygous atg12 plants fail to generate Atg8-PE adducts and poorly mobilize leaf nitrogen into seeds and are consequently hypersensitive to nitrogen starvation. Collectively, the omics profiles of WT and atg12 seedlings before and after nitrogen stress revealed central roles for autophagy in carbon and secondary product metabolism, ion uptake, the turnover of ribosomes and proteasomes, and proteolysis in general. In fact, the levels of many chloroplast-resident proteins were lower in atg12 seedlings, suggesting that impaired autophagy accelerates plastid degradation by alternative routes. atg12 plants also accumulate high levels of free fatty acids and express high levels of various phospholipases, implying that autophagy plays an unexpected but key role in maintaining lipid homeostasis. Together, these studies further increased our insights into the processes significantly impacted by autophagy, which should facilitate the development of crops which are more tolerant to nutrient stress.

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P141

Phosphate limitation reduces the kernel number in the maize ear

(submitted by Ruifeng Wang <wangruifeng@cau.edu.cn>)
Full Author List: Wang, Ruifeng[1]; Zhao, Cheng[2]; Li, Xuexian[1]
[1] Department of Plant Nutrition, China Agricultural University, Beijing 100193, China
[2] Shanghai Center for Plant Stress Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai 201602, China

Phosphorus (P) is a macronutrient required for maize development and kernel formation although underlying physiological and molecular mechanisms remain elusive. We found that P limitation significantly reduced the kernel number while not affected longitudinal growth of the ear. P limitation caused significant decreases in concentrations of soluble proteins and free amino acids, as well as variations in hormone accumulation, especially a significant decrease in IAA accumulation. Transcriptional profiling uncovered 847 genes differentially expressed, with 423 genes induced and 424 genes repressed, including various hormone signaling components and transcription factors. Expression of transporter genes were overall down-regulated. Importantly, 313 out of 847 differentially expressed genes harbored PHR1 binding sequences (P1BS). Presence of P1BS elements in promoter regions of nitrogen metabolism and amino acid transporter genes indicated potentially direct cross-talk between P and nitrogen signaling. Yeast experiments showed that amino acid transporters ZmAAP2 and ZmLHT1 function in transport of crucial amino acids. Taken together, our results suggest that the kernel abortion caused by P limitation is closely associated with variations in hormone signaling and alterations in N metabolism and amino acid transport.

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P142

Plant transformation and gene editing services at the Cornell University Plant Transformation Facility

(submitted by Matthew Willmann <mrw6@cornell.edu>)
Full Author List: Lindskoog, Zachary[1]; Willmann, Matthew R.[1]
[1] Cornell University, Plant Transformation Facility, B22 Weill Hall, Ithaca, NY 14853

The Cornell University Plant Transformation Facility (PTF) is a new service facility of the College of Agriculture and Life Sciences (CALS) and its School of Integrative Plant Science (SIPS). PTF is charged with supporting the research of plant scientists by making transgenic and gene-edited plants of time-consuming or hard-to-transform species, in addition to providing consultation on research projects and grant applications. Currently, we are focused on transforming maize (*Zea mays*) and rice (*Oryza sativa*) using *Agrobacterium tumefaciens*. For maize, we transform the inbred line B104. For rice, we offer transformation of two Japonica rice genetic backgrounds—Nipponbare and Kitaake—and one Indica rice cultivar—IR64. We are the only academic facility in the United States transforming Indica rice. We are also in the process of developing transformation methods for at least three more rice cultivars that have not been transformed previously. In the next year, we will be adding transformation services for apple (*Malus x domestica*) and wheat (*Triticum aestivum*). The typical workflow begins with the receipt of constructs from our users. While we do not make the constructs, we can provide advice on their design, particularly for constructs used for CRISPR/Cas9 gene editing. The facility then uses the constructs to make transgenic and gene-edited plants that are returned to the customer as regenerated T0 plantlets. In rare occasions, we will also consider growing these plantlets out and delivering T1 seed. For more information on the facility or to inquire about or request transformation services, please see http://sips.cals.cornell.edu/research/plant-transformation-facility or email the Director, Matthew R. Willmann, at mrw6@cornell.edu.

Funding acknowledgement: Cornell University, College of Agriculture and Life Sciences

P143

Plastome analysis of five species of parasitic plants

(submitted by Daniel Frailey <dfrailey@uga.edu>)
Full Author List: Frailey, Daniel C[1]; Chaluvadi, Srinivasa R[1]; Bennetzen, Jeffrey L[1]
[1] University of Georgia; Athens, GA 30606

The circular chloroplast genomes (plastomes) of most plants are highly conserved in gene content and gene order. Their most notable structural features are two single copy regions that flank a large region that is duplicated in an inverted orientation. Non-photosynthetic parasitic plants show a large number of rearrangements, including gene deletions. Recently, photosynthetic parasitic plants have been shown to also have rearrangements in their plastomes, although generally to a lesser extent than non-photosynthetic plants. In this study, we sequenced and annotated the plastomes from 5 photosynthetic parasitic plants, *Striga hermonthica*, *S. forbessii*, *S. aspera*, *Buchnera hispida*, and *Aureolaria virginica*. The three *Striga* species as well as *Buchnera hispida* all had increased plastome sizes. This was unexpected since many parasitic plants studied so far have decreased plastome sizes compared to non-parasitic plants. We found that the increased plastome size was due to expansion of the inverted repeat regions. We also found several other lineage-specific rearrangements, including some gene deletions.

P144

Producing reporter gene constructs for investigating the role of G-Quadruplex (G4) DNA elements in gene regulation

(submitted by Brianna Griffin <bdg13@my.fsu.edu>)
Full Author List: Griffin, Brianna D.[1]; Winn, Morgan N.[1]; Bass, Hank W.[1]
[1] Department of Biological Science, Florida State University, Tallahassee, FL, USA 32306-4295

Genes are known to be regulated by transcription factors that bind to specific cis-acting regulatory elements in and around the genes. Individual genes typically have unique combinations of cis-elements that collectively govern their expression patterns. Co-regulated genes often have nearby copies of the same element. G-quadruplex (G4) DNA is a widely distributed class of potential cis elements, prevalent in the genomes of plants, animals and bacteria, with numerous proposed genetic functions. G4 DNA refers to small, non-duplex, 4-stranded structures that fold up within or across strands of DNA. As cis elements, G4s may function as reversible elements that form under specific conditions. G4 DNA sequence motifs can be identified genome-wide using computer algorithms. G4 DNA is implicated in human cancer-related gene regulation, but relatively little is known about plant G4s. Andorf et al., (*J Genet Genomics*, 2014, DOI: 10.1016/j.jgg.2014.10.004) recently identified thousands of G4 motifs in stress-response genes of maize. To examine if and how G4 elements might modulate gene expression levels, we have made reporter gene constructs with and without maize G4s, using *hexokinase4* (*hex4*) and *shrunken1* (*sh1*, sucrose synthase) antisense 5'UTR G4 sequences. In addition, we have mapped and examined a new class of putative G4s, the double stranded G4 motifs. These ds G4 motifs are not yet characterized in plants and may be part of the maize cistrome. Understanding how maize G4s function remains a major and complex challenge that when met will offer mechanistic insights and opportunities for control of maize gene expression.

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P145

Protein-protein interaction of splicing factors involved in the processing of U12 introns in maize

(submitted by Jacob Corll <jbcorll@oakland.edu>)
Full Author List: Corll, Jacob[1]; Brigolin, Christian J.[1]; Shodja, Donya[1]; Martin, Federico[2]; Settles, A. Mark[2]; Lal, Shailesh[1]
[1] Department of Biological Sciences, Oakland University, Rochester Hills, MI 48309
[2] Horticultural Sciences Department, University of Florida, Gainesville, FL 32611

Accurate removal of introns from the primary transcript is a fundamental process of gene expression in eukaryotes. In addition to major, U2-type introns, the vast majority of eukaryotes also contain minor, U12-type introns. U12-type introns are distinct from U2-type introns and are spliced by a separate, minor spliceosome. Representing approximately 0.5% of all introns, splicing of U12-type introns plays an important role during cell differentiation and development in both plants and animals. Recent molecular characterization of two maize defective kernel mutants, *rbm48* and *rgh3*, indicates that these loci are required for the efficient splicing of U12-type introns. Both RBM48 (RNA Binding Motif48) and RGH3 (Rough endosperm3) are conserved proteins found in most eukaryotes, with RGH3 encoding the ortholog of human ZRSR2. Transient expression of fluorescent-tagged proteins in *Nicotiana benthamiana* exhibited co-localization of RBM48 with RGH3 and core splicing U2 snRNP Auxiliary Factor (U2AF) within the nuclear speckles. Furthermore, Biomolecular Fluorescence Complementation (BiFC) suggests that RBM48 physically interacts with RGH3 and the small subunit of U2 Auxiliary Factor (U2AF1). To determine if these are direct protein-protein interactions, we expressed His-tagged, recombinant maize RBM48, RGH3, and U2AF1 in *E.coli*, and raised antibodies against the proteins. *In vitro* co-immunoprecipitation and pull-down assays confirmed interactions of RBM48 with RGH3 and U2AF1. These data point to a key role for RBM48 during pre-mRNA splicing and seed development in maize.

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P146

Proteomic profiling of maize chromatin-associated proteins modulated by pathogen attack

(submitted by Maxwell McReynolds <maxwellm@iastate.edu>)
Full Author List: McReynolds, Maxwell R[1]; Walley, Justin W[1]
[1] Department of Plant Pathology and Microbiology, Iowa State University, Ames, IA 50011, USA

The genomic DNA of eukaryotes is packaged into chromatin, which controls access of DNA to transcriptional machinery as well as serving as a binding scaffold for chromatin-associated proteins. The differential recognition and subsequent binding of these chromatin-associated or "reader" proteins to various states of post-translationally modified histones has been linked to many key developmental and physiological processes. HC-toxin is a cyclic tetra-peptide effector molecule secreted by *Cochliobolus carbonum* that functions as a histone deacetylase inhibitor (HDACi) and is the virulence factor which enables successful infection of susceptible host plants. Using mass spectrometry-based acetylome profiling we have determined that treatment of susceptible corn plants with exogenous HC-toxin or virulent *C. carbonum* results in hyper-acetylation of maize histone H4 at lysine residues 5, 8, 12, and 16 (K5/8/12/16). We hypothesize that HC-toxin-induced hyper-acetylation of K5/8/12/16 on histone H4 leads to recruitment of specific host reader(s) that act to potentiate pathogen virulence. To identify these differential proteins, we are employing a peptide pull-down screen using synthesized peptides of non-acetylated and K5/8/12/16 histone tails to probe and capture reader proteins from maize nuclear extract. Once the differential reader proteins from the peptide baits have been obtained, we will utilize mass spectrometry-based proteomic profiling to identify and characterize the sets of readers that have been recruited to bind our synthesized histone baits. This approach will identify potentially novel reader proteins and set up future experiments examining downstream targets of these reader proteins through various protein and DNA interaction assays. The elucidation of both direct and downstream components of histone mark targeting by reader proteins will provide knowledge of how pathogens can modulate their host in an epigenetic matter thus possibly leading to the creation of molecular tools to counteract this virulence mechanism.

P147

Rice *Phosphorus Starvation Tolerance 1* gene and its sorghum and maize homologs improve root and vegetative growth in transgenic tobacco

(submitted by Sylvia Morais de Sousa <sylvia.sousa@embrapa.br>)
Full Author List: Lopes, Simara S[1 2]; Palhares, Patricia LS[1 3]; Lana, Ubiraci GP[1 3]; Alves, Meire C[1]; Barros, Beatriz A[1]; Magalhães, Jurandir V[1 2]; Guimarães, Claudia T[1 2]; Carneiro, Andrea Almeida[1]; de Sousa, Sylvia M[1 2 3]
[1] Embrapa Maize and Sorghum, Sete Lagoas, MG, Brazil, 35701-970
[2] Universidade Federal de São João del-Rei – UFSJ, São João del-Rei, MG, Brazil, 36307-352
[3] Centro Universitário de Sete Lagoas - UNIFEMM, Sete Lagoas, MG, Brazil, 35701-242

Low phosphorus (P) availability in soil is a major constraint for crop production in tropical regions. PHOSPHORUS-STARVATION TOLERANCE1 (OsPSTOL1) is a protein kinase that enhances root surface, P acquisition and grain yield in rice under P deficiency. Sorghum homologs of *OsPstol1* were identified by association mapping 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, in Brazil and/or in Mali. Maize and sorghum candidate genes co-localized with quantitative trait loci (QTL) for traits underlying root morphology and dry weight accumulation under low P via QTL mapping. In order to validate the function of these genes, rice *OsPstol1* (control) and its maize (ZmPSTOL3.06, ZmPSTOL8.02 and ZmPSTOL8.05_1) and sorghum (Sb07g002840, Sb03g031690 and Sb03g006765) homologs were cloned downstream of ubiquitin promoter in pMCG1005 vector, using *Bar* gene as a selective marker. Tobacco *Petit havana* plants were genetically transformed via *Agrobacterium tumefaciens* EHA101 strain and regenerated from selected callus in shooting and rooting medium supplemented with 100 mg/ml of Tioxin and 1 mg/L of Phosphinothricin. PCR with gene specific (~700 bp) and Bar (~400 bp) primers confirmed the presence of *Pstol1* genes in tobacco plants. Several plants presented one copy of the trangene, and those that also showed overexpression of the transgene were selected for evaluation under low P conditions. Overexpression of *Pstol1* genes significantly enhanced vegetative plant growth and root surface area on low P, indicating that these genes act in a similar manner to *osPstol1* gene in rice plants.

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P148

Evogene, plant innovation and current maize applications

(submitted by Schushan, Maya <Maya.Schushan@evogene.com>)
Full Author List: Evogene Ltd.[1]
[1] Evogene Ltd, 13 Gad Feinstein st., Rehovot 7612002, Israel.

Evogene is a leading biotechnology company for the improvement of crop productivity for the food, feed and fuel industries. The company has developed an innovative technological platform, which allows to utilize big-data driven predictive biology for improving crop productivity, focusing on the discovery and optimization phases of product development. The company utilizes its capabilities to bring innovation to three types of agriculture products: improved seed traits (addressing yield, tolerance to environmental stresses and resistance to insects and diseases); innovative ag-chemicals (with current focus on novel herbicide solutions for weed control); and ag-biologicals (biostimulants and biopesticides). Evogene has collaborations with world-leading seed and ag-chemical companies.
Among other crops, Evogene is applying its platform for improving maize crop productivity, currently developing the following innovative products: i) Transgenic maize products in collaboration with Monsanto, focusing on yield, abiotic stress and Fusarium ii) Insect control traits, for which toxins were validated for activity against Lepidoptera insects iii) Non-selective herbicides, for which new targets were discovered and validated, and recently reported chemical hits displaying herbicidal activity iv) Ag-Biological microbiome-based biostimulants, for which various candidates demonstrated significant target traits and yield improvement in recent maize field trials.

P149

Seteria viridis as a model for pathogen resistance in the *Poaceae*

(submitted by Charles Hunter <charles.hunter@ars.usda.gov>)
Full Author List: Hunter, Charles T[1]; Christensen, Shawn A[1]; Rering, Caitlin[1]; Block, Anna K[1]; Alborn, Hans T[1]
[1] USDA-ARS; Chemistry Research Unit; Center for Medical, Agricultural and Veterinary Entomology; Gainesville, FL, 32608

Seteria viridis is an effective model system for functional genetics in the C4 Poaceae grasses, which include important crops like maize, sorghum, and sugar cane. The small genome size, short stature, rapid life cycle, and the availability of genetic transformation protocols, make Seteria an attractive organism for molecular genetic studies. Here we assess the strength of Seteria as a model for studying fungal resistance in C4 grasses by comparing the chemical defense responses between Seteria and maize during pathogen infection. Chemical defenses amongst even closely-related plant species often vary widely. This is especially true for antimicrobial secondary metabolites such as the terpenoid phytoalexins and benzoxazinoids, some of which have only been identified in a single plant species. It is therefore imperative that we understand the similarities and differences between our primary species of interest and a proposed model species for conducting functional genetics. Here we tested whether the common maize pathogen, *Cochliobolus heterostrophus* (Southern Leaf Blight, SLB) would also infect Seteria. Under controlled conditions, SLB showed similar growth and infection rates on Seteria and maize. Using this system, we measured defense-related phytohormones and diverse direct defense metabolites in SLB-infected tissues of Seteria and maize. Here we discuss the similarities and differences in chemical defense responses to pathogen infection. Our findings suggest that Seteria will provide an effective model species for conducting functional genetics on many of the chemical defense pathways we examined.

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P150

Soil-mediated cover crop effect on corn defense responses to black cutworm

(submitted by Shan Jin <szj133@psu.edu>)
Full Author List: Jin, Shan[1 2]; Luthe, Dawn S.[1 2]; Murrell, Ebony G.[3]; Kaye, Jason P.[3]; Barbercheck, Mary E[4]
[1] Intercollegiate Graduate Program in Plant Biology, The Pennsylvania State University, University Park, PA 16802
[2] Department of Plant Science, The Pennsylvania State University, University Park, PA 16802
[3] Department of Ecosystem and Science Management, The Pennsylvania State University, University Park, PA 16802
[4] Department of Entomology, The Pennsylvania State University, University Park, PA 16802

Black cutworm, *Agrotis ipsilon* (BCW) is a problem for organic farming systems using cover crops as the main avenue in managing soil fertility. BCW adults oviposit in nearby cover crops in early spring and young larvae establish on plant materials and migrate to emerging corn seedlings for feeding. Cover crop effects on soil may affect plant defense responses to insects in following cash crops. We performed both field and greenhouse experiments for two years to examine whether cover crops affect corn resistance to BCW and whether the resistance relates to the expression of herbivore defense genes in corn. We hypothesize that corn grown in soil from the three (3SppN) and six (6Spp) species cover crop mixtures will retard BCW growth and have more robust defense responses compared to the corn grown in fallow soil.
Insect bioassays showed that in 2014, BCW fed on 3SppN corn had the highest growth because 3SppN cover crops elevated soil nitrogen level. In 2015, BCW growth was opposite of the trend in 2014. Secondly, the expression of four corn defense genes was measured in plants grown in soil from the cover crop plots. In 2014, 3SppN corn accumulated more *aos* mRNA than 6Spp and fallow corns; while there was no difference in *rip2* mRNA levels among cover crop treatments. In 2015, 6Spp corn accumulated more *rip2* and *tps23* mRNA; while there were no differences in *aos* and *mpi* mRNA levels.
This is the initial step investigating the molecular mechanisms of soil-mediated cover crop effects on corn defense responses to BCW. The whole system has four levels of players in interactions: cover crop - soil - corn plant - BCW. It has opened the gate for illuminating the mechanisms contributing to changes in overall arthropod pest resistance that occur when crops are grown under organic management.

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P151

Specificity and function of the maize root hair transcriptome

(submitted by Stefan Hey <stefan.hey@uni-bonn.de>)
Full Author List: Hey, Stefan[1]; Baldauf, Jutta[1]; Opitz, Nina[1]; Lithio, Andrew[2]; Pasha, Asher[3]; Provart, Nicholas[3]; Nettleton, Dan[2]; Hochholdinger, Frank[1]
[1] INRES, Crop Functional Genomics, University of Bonn, 53113 Bonn, Germany
[2] Department of Statistics, Iowa State University, Ames, Iowa 50011-1210, USA
[3] Department of Cell and Systems Biology, University of Toronto, Toronto, ON, M5S 3B2, Canada

Root hairs are important for water and nutrient acquisition by increasing the root surface. Most of our knowledge about the molecular basis of root hair development was obtained in the model species Arabidopsis. In contrast, only little is known about the mechanisms underlying root hair development in monocots such as maize. To date, six mutants (*rth1* - *rth6*) with impaired root hair elongation have been identified in maize. In the present study the transcriptome of maize root hairs was dissected. Transcriptome profiling demonstrated that the single cell-type root hair transcriptome was less complex than the transcriptome of multi cell-type primary roots without root hairs. Functional GO-terms enriched among genes preferentially expressed in root hairs were mainly involved in energy metabolism. Phylogenetic analyses in combination with transcriptome profiling indicated common features, but also showed differences in the genetic regulation of root hair development. Finally, a maize root view of the eFP browser was implemented including the root hair transcriptome of the present study and several previously published maize root transcriptome datasets. The eFP browser provides color-coded expression levels for these root types and tissues for any gene of interest thus providing a novel resource to study gene expression and function in maize roots.

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P152

Suppression of *CINNAMOYL-CoA REDUCTASE* increases the level of monolignol-ferulates in maize lignins

(submitted by Steven Karlen <skarlen@wisc.edu>)
Full Author List: Smith, Rebecca[1,2]; Cass, Cynthia[3]; Mazaheri, Mona[1,4]; Sekhon, Rajandeep[4]; Heckwolf, Marlies[4]; Kaeppler, Heidi[4]; de Leon, Natalia[1,4]; Mansfield, Shawn[5]; Kaeppler, Shawn[1,4]; Sedbrook, John[3]; Karlen, Steven[1,2]; Ralph, John[1,2]
[1] University of Wisconsin-Madison, Department of Energy Great Lakes Bioenergy Research Center, the Wisconsin Energy Institute, Madison, WI USA 53726
[2] University of Wisconsin-Madison, Department of Biochemistry, Madison, WI USA 53706
[3] Illinois State University, Department of Energy Great Lakes Bioenergy Research Center, School of Biological Sciences, Normal, IL USA 61790
[4] University of Wisconsin-Madison, Department of Agronomy, Madison, WI USA 53706
[5] University of British Columbia, Department of Wood Science, Vancouver, BC, Canada

One of the most promising alternatives to fossil fuels involves generating ethanol (or second-generation biofuels) from plant cell wall polysaccharides (sugars), such as cellulose and hemicelluloses. The problem with this strategy is that the production of cellulosic ethanol is not yet as efficient or cost-effective as fossil fuels. The main reason for this is the harsh treatments required to remove the recalcitrant cell wall phenolic polymer lignin in order to access the cell wall sugars. For this reason, decreasing or altering lignin content in potential biofuel crops has received a lot of attention from the scientific community. Lignin, though detrimental to biofuel processing, is essential to the support and defense of the plant body and decreases in lignin content are therefore usually associated with pendant stems, increased susceptibility to pathogens and reduced biomass yield. The digestibility of the cell walls can be improved by introducing labile ester bonds, which are broken under weak base treatment at room temperature, into the lignin backbone. The FERULOYL-CoA MONOLIGNOL TRANSFERASE (FMT) enzyme, which is naturally found in many plants, uses feruloyl-CoA and monolignols to synthesize the ester-linked monolignol ferulate conjugates. A mutation in the first lignin-specific biosynthetic enzyme, CINNAMOYL-CoA REDUCTASE (CCR1), results in an increase in the pool of feruloyl-CoA. Maize (*Zea mays*) has a native putative FMT enzyme, and its *ccr1* mutants produced an increased pool of feruloyl-CoA that was used for conversion to monolignol ferulate conjugates. The increase in conjugates correlated with an improvement in the digestibility of maize stem rind tissue.

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P153

Testing the role of Sorghum 3-deoxyanthocyanidin Phytoalexins as potential biopesticides in combating foliar diseases in *Zea mays*

(submitted by Cullen Dixon <cwd5317@psu.edu>)
Full Author List: Dixon, Cullen W.[1]; Gaffoor, Iffa[1]; Chopra, Surinder[1]
[1] Pennsylvania State University, The College of Agricultural Sciences, Department of Plant Science, University Park, PA 16802

Maize, *Zea mays*, is one of the world's top commodities for food, feed, and fuel. Protecting this precious staple is a top priority of agriculturalists and officials globally. One of the major threats to the health of *Z. mays* is due to anthracnose stalk rot, and occasionally, anthracnose leaf blight. It is caused by the fungus *Colletotrichum graminicola*, a pathogen specific to *Z. mays*. The sorghum plant, *Sorghum bicolor*, produces 3-deoxyanthcyanidin flavonoids (3-DA) that have been shown to act as phytoalexins. These 3-DAs include apigeninidin, luteolinidin, and other derivatives of varying toxicity. It has been shown in sorghum that these flavonoids are produced upon fungal ingress within the vesicles of infected or nearby cells. These vesicles release their toxic contents killing both the cell and the infecting fungal hyphae. The composition of 3-deoxyanthiocyaidins produced is dependent upon the *S. bicolor* genotype, with some producing larger proportions of the greater toxicity compounds than others. The individual 3-deoxyanthocyanidin compounds have been shown to effectively combat *Colletotrichum sublineolum*, the causal pathogen of anthracnose leaf blight in sorghum, by Snyder and Nicholson (1990). These flavonoid compounds have been shown *in vitro* to inhibit both *C. graminicola* spore germination, and slow or cease any further proliferation of the hyphae, thereby killing the fungus. Therefore, they have the potential to become a highly effective, sustainable, and natural solution to combating fungal infections of *Colletotrichum* in *Z. mays*. *In vitro* assays were performed using flavonoid extracts from two different sorghum genotypes to determine their toxicity on *C. graminicola* spores. Subsequently, *in vivo* assays were performed by treating infected *Z. mays* plants with the noted 3-DA extracts to test these compounds as a potential natural, plant based, commercially-applicable fungicides. Preliminary results in maize genotype B73 are promising within a greenhouse setting.

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P154

The BHLH type transcription tactor ZmCHS10 mediates low temperature response in maize

(submitted by Jingyan Liu <liujingyan826@qq.com>)
Full Author List: Liu, Jingyan[1]; Yang, Shuhua[1]
[1] State Key Laboratory of Plant Physiology and Biochemistry, College of Biological Sciences, National Plant Gene Research Center, China Agricultural University, Beijing, China,100193

Cold stress is one of the major factors to limit the yield and quality in maize. To identify the important components involved in cold response, we screened a maize candidate library including hundreds of overexpression lines and CRISPR-generated knock out mutants. Here we report ZmCHS10, a maize BHLH type transcription factor, play important roles in cold response and developmental process. The knockout mutants of ZmCHS10 conferred chilling sensitive phenotype at the seedling stage, and also displayed developmental defects in the later stage. RNA-Seq analysis showed that 1390 differentially expressed genes (DEGs) were ZmCHS10-regulated. After cold treatment, 1196 DEGs were regulated by ZmCHS10. GO analysis showed that these genes cover a wide range functions involved in abiotic stress, metabolic progress, catalytic activity, etc. ZmCHS10 can bind some cold response targets and up-regulate gene expression as it does in Arabidopsis. Overexpression of ZmCHS10 partially rescued the phenotype of atchs10 mutant, suggesting that function of ZmCHS10 partially overlapped with AtCHS10. These results demonstrate the important roles of ZmCHS10 in cold response and developmental process, giving the new clues for understanding the pleiotropic biological function of ZmCHS10.

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P155

The ligand of FERONIA receptor-like kinase (RLK), Rapid Alkalinization Factor 1 (RALF1), functions in stomatal movement regulation via G protein signaling

(submitted by Yunqing Yu <yyu@danforthcenter.org>)
Full Author List: Yu, Yunqing[1]; Assmann, Sarah M.[2]
[1] Donald Danforth Plant Science Center, St. Louis, MO 63132
[2] Biology Department, Penn State University, University Park, PA 16802

Heterotrimeric guanine nucleotide-binding (G) proteins are composed of Gα, Gβ, and Gγ subunits and function as molecular switches in signal transduction. In *Arabidopsis thaliana*, there are one canonical Gα (GPA1), three extra-large Gα (XLG1, XLG2 and XLG3), one Gβ (AGB1) and three Gγ (AGG1, AGG2 and AGG3) subunits. Previous studies have shown that Arabidopsis G proteins are involved in numerous processes related to plant development and environmental response. To identify AGB1 interactors, we performed co-immunoprecipitation (co-IP) and mass spectrometry using transgenic Arabidopsis expressing *35S::FLAG-AGB1* in the *agb1-2* mutant background, with wild-type Col plants as a control. Plasma membrane protein-enriched fractions obtained using two-phase partitioning method were used for the co-IP assays. After eliminating proteins present in the control IP, commonly identified contaminants, and organellar proteins, a total of 103 candidate AGB1-associated proteins were confidently identified. We identified almost all of the G protein subunits, receptor-like kinases (RLKs), Ca^{2+} signaling related proteins including Calmodulin 2, Annexin D4 and IQ-domain 31 protein, and 14-3-3-like proteins, which may couple with G protein signaling. We further confirmed physical interaction between AGB1 and one of the RLK candidates, FERONIA (FER), using BiMolecular Fluorescence Complementation (BiFC) assays. RALF is a polypeptide ligand of FER and promotes phosphorylation of FER and other proteins. Previous studies show that RALF1 increases external pH and inhibits growth in seedlings. In this study, mutant analysis revealed that RALF1 also play roles in stomatal movement regulation, inhibiting stomatal opening and promoting stomatal closure. We further showed that AGB1, AGGs and XLGs, but not GPA1, participate in these processes.

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P156

The maize *mat1* mutant alters mitochondrial respiratory chain and leads to an empty-pericarp phenotype

(submitted by Peng Liu <mcliup@ufl.edu>)
Full Author List: Liu, Peng[1]; Saunders, Jonathan[1]; Lundgren, Jennifer[1]; Wu, Shan[1]; McCarty, Donald[1]; Koch, Karen[1]
[1] Horticultural Sciences Department, and Plant Molecular and Cellular Biology Program, University of Florida, Gainesville, FL 32611

The nuclear genome of maize includes multiple genes for group-II intron maturases. These are targeted to mitochondria where they bind directly to gourp II introns and mediate splicing of mitochondrial pre-mRNAs. These maturase genes are thought to derive from the mitochondrial genome and encode ancient splicing factors. Similar functions have also evolved more recently for other splicing factors such as the PPRs. By screening the UniformMu population, we obtained transposon-insertion mutants for several maturase genes, *Mat1* among them. The *mat1* mutant has an empty pericarp (ep) phenotype. Although kernels are infertile and the phenotype is dramatic, both endosperm and embryo progress through initial stages of development without critical aspects of mitochondrial function. The *mat1* mutant shows deficient splicing of mitochondrial mRNAs, including those for complex I of the respiratory chain. Through comparison of transcriptome profiles from the *mat1* and wild-type kernels, we found that mutant material lacked normal levels of mRNAs for biosynthesis of both starch and storage proteins. Also, transcripts for the entire respiratory chain were up-regulated in the *mat1* mutant, possibly due to the feedback input. Interestingly, genes encoding cytochorome P450s, glutathione-S-transferases, and UDP-glycosyltransferases were also highly induced at the mRNA level. These enzymes are prominent contributors to detoxification, so their up-regulation could be advantageous if dysfunctional respiration leads to elevated ROS and stress metabolites from mitochondria. MAT1 is thus essential to normal kernel development through its role in splicing introns of the mitochondrial genome.

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P157

The nuclear pore complex component *aladin* is necessary for tassel architecture and asymmetric cell division in maize

(submitted by Norman Best <nbbest@purdue.edu>)
Full Author List: Best, Norman B.[1]; Addo-Quaye, Charles[2]; Schulz, Burkhard[1]; Johal, Guri[3]; Dilkes, Brian P.[4]
[1] Department of Horticulture and Landscape Architecture, Purdue University, West Lafayette, IN, USA 47907
[2] Division of Natural Sciences and Mathematics, Lewis and Clark State College, Lewiston, ID, USA 83501
[3] Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN, USA 47907
[4] Department of Biochemistry, Purdue University, West Lafayette, IN, USA 47907

A recessive mutant with shortened upper internodes, altered tassel architecture, and aberrant asymmetric division of stomatal subsidiary cells was identified in a B73-background EMS population of maize. Using linkage mapping and high throughput sequencing, we identified a nonsense mutation in the last exon of the nuclear pore complex (NPC) subunit *aladin* (*ali*), as the cause of these phenotypes. The NPC regulates movement of macromolecules between the nucleus and cytoplasm and connects the nucleus to the cytoskeleton. A targeted mutagenesis screen produced a second allele, *ali-2*, which encodes a nonsense mutation in the 10th exon and confirmed the identity of the gene. The *ali-2* homozygous mutants are severe dwarves that fail to develop reproductive structures. The *ali-2* allele was semi-dominant in *ali-1*/*ali-2*, as plants were significantly shorter than *ali-1* homozygous plants. The proportion of aberrant stomata was not discernably different in *ali-1*/*ali-2* and *ali-2* homozygous plants demonstrating that *ali-1* was haploinsufficient for stomatal aberrancy in the presence of the *ali-2* allele. Thus, *ali-1* was recessive to both the WT and the *ali-2* nonsense allele, but conditioning greater and lesser degrees of stomatal aberrancy, respectively. Analysis of differential mRNA accumulation identified up-regulation of other NPC components in *ali-1* tassels but no difference in ALI transcript abundance as compared to WT. The intermediate phenotype of the *ali-1* mutant was used to identify alleles that suppress or enhance the mutant phenotype. The F2 generation of crosses between *ali-1* and 25 different inbreds was phenotyped. Dramatic enhancement of the mutant phenotype including variably penetrant lethality, up to 90% reduction of plant height, and similar plant appearance to *ali-2* homozygotes segregated in *ali-1* X M37W F2 families. These findings demonstrate that *ali* is an essential gene, affects tassel architecture and asymmetric cell division, and detects maize standing variation in NPC function.

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P158

The silk surface lipid metabolome responds to abiotic stress and offers protection against desiccation

(submitted by Bri Vidrine <bvidrine@iastate.edu>)
Full Author List: Vidrine, Bri[1 2]; Sievers, Zachary[1]; Westgate, Mark[3]; Lauter, Nick[1 4]; Nikolau, Basil[1 2]; Yandeau-Nelson, Marna[1 5]
[1] Interdepartmental Genetics Graduate Program; Iowa State University, Ames, IA, 50011
[2] Roy J. Carver Department of Biochemistry, Biophysics & Molecular Biology, Ames, IA, 50011
[3] Department of Agronomy; Iowa State University, Ames, IA, 50011
[4] USDA-ARS Corn Insect and Crop Genetics Research Unit, Ames, IA, 50011
[5] Department of Genetics, Development and Cell Biology; Iowa State University, Ames, IA, 50011

Maize silks are essential for pollen reception and hydration, pollen tube growth and fertilization, and thus corn yield. While large silk surface areas are advantageous for capturing wind-carried pollen grains, surfaces are also susceptible to water loss, especially under desiccating conditions. As a primary line of defense, the silk cuticle is coated with a hydrophobic barrier of extracellular surface lipids (SLs) that is exceptional in its high hydrocarbon composition (~90% of total SLs in inbred B73). To consider the effect of abiotic stress on surface lipid accumulation, B73 silks were harvested from growth chamber-grown plants exposed to water deficit treatments under two different temperature treatments (25 or 30°C) imposed after tassel emergence. Results of subsequent silk surface lipid profiling demonstrate that the concentration of unsaturated hydrocarbons increase in response to temperature stress, whereas saturated hydrocarbons increase in response to water deficit. In a separate experiment, we tested the protective capacity of the SL metabolome against desiccation by measuring rates of water loss for excised silks from four diverse inbred lines (including B73) subjected to four combinations of temperature and humidity treatments. When excised silks were subjected to 15% relative humidity, the rates of water loss from inbred (MO378) were ~1.5 to 2-fold faster than silks from B73. In contrast, water loss rates did not vary significantly between inbreds subjected to 85% RH. Statistical modeling incorporating both water loss rates and surface lipid composition suggest that saturated hydrocarbons do provide some level of protection from water loss. Collectively, our initial experiments demonstrate that silk SLs respond to abiotic stress and that these SLs likely offer protection from desiccation, however, other potential causal factors will also be discussed.

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

P159

Trait development process: dicamba, glufosinate tolerant corn

(submitted by Rita Varagona <rita.j.varagona@monsanto.com>)
Full Author List: Goley, Mike[1]; Huang, Jintai[1]; Sparks, Oscar[1]; Shao, Aihua[1]; Stoecker, Martin[1]; Groth, Mark[1]; Klingaman, Tracy[1]; Martino-Catt, Susan[1]; Varagona, Rita[1]
[1] Monsanto Company, 700 Chesterfield Parkway West, Chesterfield, MO, USA, 63017

Monsanto is developing dicamba and glufosinate tolerant corn event MON 87419 for commercialization by the end of the decade. This presentation will focus on the early development process (Phase 2) from transformation to the selection of commercial event MON 87419. Rigorous molecular characterization, event selection, and field testing resulted in an event with exceptional herbicide tolerance and agronomic equivalence. The Phase 2 processes not only demonstrate trait performance, but also ensure safety and minimize the risk of unintended effects.

P160

Transcriptomic dissection of heterosis in maize primary roots at the interface of genotype and development

(submitted by Jutta Baldauf <baldauf@uni-bonn.de>)
Full Author List: Baldauf, Jutta A.[1]; Marcon, Caroline[1]; Lithio, Andrew[2]; Nettleton, Dan[2]; Hochholdinger, Frank[1]
[1] INRES, Crop Functional Genomics, University of Bonn; 53113 Bonn, Germany
[2] Department of Statistics, Iowa State University; Ames, IA 50011-1210, USA

Distantly related maize inbred lines exhibit an exceptional degree of structural genomic diversity, which is probably unique among plants. Heterozygous F_1-hybrid progeny of such inbred lines are often more vigorous than their homozygous parents, a phenomenon known as heterosis. We aim to extend and generalize our previous observations of transcriptomic patterns made in reciprocal hybrids of the inbred lines B73 and Mo17 to a more diverse panel of maize inbred lines and their hybrid progeny. For this purpose we investigate how the genetic divergence of seven selected parental inbred lines (B73, Mo17, A554, H84, H99, Oh43, W64A) is reflected in the transcriptomic landscape of primary roots of their hybrid progeny during development. A RNA-seq experiment was designed to maximize the number of direct comparisons among the parent-hybrid pairs and simultaneously to ensure a high degree of precision for indirect comparisons. The quality trimmed paired-end sequencing reads were aligned to the third version of the maize reference genome. Based on normalized read counts, 28,593 genes were determined to be active among all genotypes at the three developmental stages of primary roots. Subsequently, we determined differential, non-additive and allele-specific expression patterns. Moreover, as an extreme form of complementation on the gene expression level in the hybrid we determined single-parent expression (SPE) patterns. SPE describes the observation that a gene is expressed in the hybrid but only in one of its two parental inbred lines. Such genes might be associated with early developmental manifestation of heterosis in primary roots of maize.

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P161

Using CRISPR-Cas9 mutagenesis to identify the maize SWEET transporters responsible for loading sucrose into the phloem

(submitted by Benjamin Julius <btjg2d@mail.missouri.edu>)
Full Author List: Julius, Benjamin[1]; Lee, Hyeyoung[2]; Cody, Jon[3]; Birchler, James[3]; Zhang, Zhanyuan[2]; Braun, David[1]
[1] Division of Biological Sciences, Interdisciplinary Plant Group, Missouri Maize Center, University of Missouri, Columbia, MO USA 65211
[2] Plant Transformation Core Facility, Division of Plant Sciences, University of Missouri, Columbia, MO USA 65211
[3] Division of Biological Sciences, University of Missouri, Columbia, MO USA 65211

Plants synthesize sucrose in their leaves, and must transport the sugar through the veins to support the growth and development of various non-photosynthetic tissues. This long-distance transport occurs in the phloem tissue in the vein. In maize leaves, sucrose is synthesized in photosynthetic cells before travelling through plasmodesmata into cells adjacent to the phloem, where it is exported into the cell wall. The ZmSUT1 sucrose transporter then imports the sucrose into the phloem companion cell, after which sucrose enters the sieve elements through plasmodesmata for long-distance transport. It has been shown in Arabidopsis, which utilizes the same transport pathway, that members of the recently identified SWEET transporter family are responsible for the initial export of sucrose to the cell wall prior to companion cell uptake. In the Arabidopsis *atsweet11; atsweet12* double mutant, phloem loading was inhibited, resulting in excess carbohydrates in the leaves. Through phylogenetic and expression analyses, the orthologous genes hypothesized to be candidates for this transport step in maize are *ZmSWEET13a*, *ZmSWEET13b*, and *ZmSWEET13c*. To determine whether these genes function in sucrose transport, two separate CRISPR-Cas9 guide RNAs (gRNA) were designed to simultaneously target all three genes and generate knockout mutations. The two gRNA constructs were independently transformed into Hi2AxB embryos. Interestingly, we observed that the two gRNAs showed different efficiencies in generating mutations. Excitingly, the leaves of the T_0 plants exhibited chlorosis, anthocyanin and carbohydrate accumulation, and displayed delayed growth, suggesting that the mutated *SWEET13* genes are responsible for sucrose phloem-loading in maize. Ongoing research is testing this hypothesis and will determine the subcellular localization and kinetic properties of these transporters.

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P162

Utilization of a split-root system for controlled, reproducible imposition of water deficit on maize seedlings

(submitted by Rachel Mertz <mertzr@missouri.edu>)
Full Author List: Mertz, Rachel A.[1]; Greeley, Laura A.[2]; Riggs, Kara J.[3]; Niehues, Nicole D.[3]; McCubbin, Tyler[1]; Braun, David M.[1]; Fritschi, Felix B.[3]; Sharp, Robert E.[3]
[1] Division of Biological Sciences, Interdisciplinary Plant Group, University of Missouri; Columbia, MO, USA 65211
[2] Department of Biochemistry, Interdisciplinary Plant Group, University of Missouri; Columbia, MO, USA 65211
[3] Division of Plant Sciences, Interdisciplinary Plant Group, University of Missouri; Columbia, MO, USA 65211

Drought is the major limiting factor for agricultural production worldwide, and improved crop varieties that maintain yields under water deficit are imperative to feed the estimated 9 billion global population by midcentury. In maize and other cereals, most water is acquired by whorls of nodal roots that develop sequentially from subterranean stem nodes rather than by seedling (primary and seminal) roots. As 85% of domestic maize experiences drought stress within a growing season, nodal roots frequently must emerge and elongate through very dry topsoil to access water at depth. The molecular genetic mechanisms of nodal root elongation maintenance at tissue water potentials that inhibit leaf and stem growth remain largely uncharacterized. We utilized a split-root system to impose precise, constant water deficits on seedling and nodal roots of the maize inbred lines FR697 and B73. Two water deficit regimes were imposed: nodal root growth at low soil water potential with well-watered seedling roots (moderate stress), and both nodal and seedling root growth at low soil water potential (severe stress). Under both stress regimes, FR697, but not B73, completely maintained node 2 root elongation. Intriguingly, although leaf expansion was reduced and tissue water potential declined under severe stress, leaves of FR697 seedlings did not wilt. In contrast, leaves of B73 showed greater wilting but less growth inhibition. Thus, FR697 exhibited greater enhancement of root-to-shoot biomass ratio under stress, and in addition, nodal root length per unit mass was attenuated, suggesting enhanced carbon partitioning to roots and altered root anatomy. To investigate the role of carbon partitioning in nodal root elongation and osmotic adjustment, we identified candidate genes from several sugar transporter families. These candidates will be mutated by targeted reverse genetics and evaluated for nodal root phenotypes using the split root system.

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P163

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

(submitted by Marcel Baer <Marcel.Baer@uni-bonn.de>)
Full Author List: Baer, Marcel[1]; Taramino, Graziana[2]; Multani, Dilbag[2]; Hochholdinger, Frank[1]
[1] INRES, Crop Functional Genomics, University of Bonn, 53113 Bonn, Germany
[2] Pioneer Hi-Bred International Inc., Johnston, IA 50131, USA

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 embyonic primary and seminal roots during early postembryonic development (Husakova et al., 2013). Moreover, the *lrt1* mutant forms additional brace roots at higher nodes compared to wild type plants. 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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P164

Zthi2 and *ZTHI3*: Prospects in breeding and benetic engineering for thiamine biosynthesis and accumulation

(submitted by Temitope Salaam <topesalaam@gmail.com>)
Full Author List: Salaam, Temitope O[1 2]; Taiwo, Idowu A[2]; Adekoya, Khalid O[2]; Omidiji, Olusesan[2]
[1] Biotechnology Department, Federal Institute of Industrial Research, Oshodi, Lagos, Nigeria. 100261
[2] Department of Cell Biology and Genetics, University of Lagos, Yaba, Lagos, Nigeria. 6457

Tropical *Zea mays* contain significant levels of thiamine, a critical vitamin required for growth and development in living organisms. Thiamine biosynthetic has been studied in several organisms including *Arabidopsis*, *Zea mays* and *Escherichia coli*. The genes involved in *Zea mays* are *Z-thi1* and *Z-thi2*, *ZTHIC*, *ZTHI3*, *Ztpk1* and *Ztpk2* which code for thiazole synthase, pyrimidine synthase, pyrimidine-phosphate kinase/thiamine mono-phosphate synthase (TMPS) and thiamine pyrophosphokinase respectively. Therefore, it is necessary to determine interrelationships that may exist between these biosynthetic genes in relation to metabolite contents and enzyme activities in *Zea mays* varieties for thiamine breeding programme. Forty-one *Zea mays* inbred lines were used in this study and a combination of biochemical and molecular biology techniques (HPLC and spectrophotometry qRT-PCR, SDS-PAGE, Western blotting and sequencing) were employed. We report a significant positive correlation exists between *Zthi2* expression and seed thiamine accumulation thus making it a "candidate" gene for seed thiamine bioaccumulation in *Zea mays*. The *ZTHI3* gene had the highest expression (2.13 - 4.23) but its corresponding enzyme, TMPS, had the least activity (0.080 - 1.424 pmol.mg⁻1protein.min⁻1). Western blot analysis detected the presence of both active di-meric (95 kDa) and inactive mono-meric (55 kDa) forms of the TMPS protein suggesting a post translational mechanism of regulating its activity. Our results further suggest that TMPS is a rate limiting enzyme in the thiamine biosynthetic pathway.

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P165

Effect of dsRNA in the control of the maize pests *Rhopalosiphum maidis* and *Schizaphis graminum*

(submitted by Newton Carneiro <newton.carneiro@embrapa.br>)
Full Author List: Carneiro, Newton P[1]; Barros, Beatriz A[1]; Alves, Meire C[1]; Noda, Roberto W[1]; Verdolin, Ana Laura M[1]; Carneiro, Andrea A[1]
[1] Embrapa Maize and Sorghum, Sete Lagoas, MG, Brazil, 35701-970

Maize (*Zea mays* L.) is one of the most important crops in the world. However, infestation by insect pests can lead to large losses of grain production, and studies on the development of alternative technologies that will control such attacks is essential. Interference RNA (RNAi) is a biological process that occurs in all eukaryotic cells that allows the silencing of genes at the post-transcriptional level, decreasing the activity of specific genes. One great potential of the RNAi application in the agriculture pests control is delivery dsRNA molecules by feeding capable to inhibit vital genes expression in the target insect pest. Once it happens it might cause insect losses of fitness or death. Aphids such as *Rhopalosiphum maidis* and *Schizaphis graminum* are considered one of the major pests in grasses such as maize and sorghum. This work aimed to identify genes in these two species that can be used by interfering RNA technology as a target for its control. The strategy was to sequence and identify expressed genes in *R. maidis* and *S. graminum* based on tests already performed on other insects, design specific gene primers to isolate T7 containing PCR fragments for transcription reaction and fragments for qPCR reaction, identify one housekeeping gene as reference for qPCR and perform biological assay using known dsRNA concentrations and time exposure. In this work, it was tested the effect of inhibition of expression of two genes (one for each specie) that the corresponding dsRNAs were capable to inhibit at least 20% of the target genes expression. However, such percentage inhibition was not capable to increase mortality rate (5 days exposure, 500 ng dsRNA, diet changed every other day) compared to the control treatment (without dsRNA). The results obtained in this work provide information of promising genes that can be used involving the interfering RNA technique in control of the maize pests *R. maidis* and *S. graminum*.

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P166

Effects of different levels of water stress on the ecophysiological characterization of *Sorghum bicolor* genotypes

(submitted by Newton Carneiro <newton.carneiro@embrapa.br>)
Full Author List: Magalhães, Paulo C[1]; Simeone, Maria L F[1]; Carneiro, Newton P[1]; Junior, Carlos C G[2]; Souza, Thiago C[3]; Oliveira, Antonio C[1]
[1] Embrapa Maize and Sorghum, Sete Lagoas, MG, Brazil, 35701-970
[2] Universidade Federal de São João del Rei, Sete Lagoas, MG, 35701-970
[3] Universidade Federal de Alfenas, Alfenas, MG, Brazil, 35130-000

The sorghum plant tolerates more water deficit than most cereals, however, during the reproductive stage, water stress is still one of the most limiting factors on this crop. The objective of this work was to describe the effects of several levels of water stress on ecophysiological characteristics of sorghum and associate them with drought tolerance mechanisms in this crop. Two contrasting drought tolerance sorghum genotypes were used: 9910032 (tolerant) and 9618158 (sensitive) and the experiment was conducted under greenhouse conditions at Embrapa Maize and Sorghum. In pre-flowering, four levels of stress were imposed on both genotypes: mild (four days of stress); medium (eight days); severe (12 days) and a last treatment that corresponds to the evaluation of the plants after seven days that the stress has been stopped. For each level, it was used the non-stressed plants treatment as control. It was evaluated: the relative chlorophyll content, Fv / Fm ratio, stomatal conductance, water potential and grain weight. Sensitive and tolerant genotypes show lower stomatal conductance for all types of stress when compared to irrigated treatments. The leaf water potential was different in medium and severe stresses. The grain mass for the sensitive genotype was higher in the irrigated treatments. On the other hand, for the tolerant genotype the stresses did not result in statistically significant differences between irrigated versus stressed. The results will be important to help in the breeding program and understand mechanisms involved in drought tolerance. A comparison analysis will be done in a similar work with maize contrasting drought tolerance genotypes.

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P167

Validation by qPCR of differentially expressed genes in maize in response to water stress

(submitted by Newton Carneiro <newton.carneiro@embrapa.br>)
Full Author List: Carneiro, Newton P[1]; Barros, Beatriz A[1]; Magalhães, Paulo C[1]; Noda, Roberto W[1]; Carneiro, Andrea A[1]
[1] Embrapa Maize and Sorghum, Sete Lagoas, MG, Brazil, 35701-970

Water stress is one of the main factors limiting agricultural production. Water deficit reduces the yield of the crop mainly in the reproductive stages. Maize is an important crop for a global food security that is being widely cultivated in tropical and subtropical areas under rain fed conditions. Drought tolerance is a complex feature and affects how gene responses in many biological processes in plants. Characterization of genes that are differentially expressed in tolerant and sensitive maize genotypes under different stress conditions might help elucidate some aspect of drought mechanism in this crop. In this work, the level of gene expression of contrasting maize species was compared for water stress tolerance and two water conditions by RNAseq and qPCR. The total RNA was extracted from roots of more tolerant maize plants, in the presence (50% less irrigated) and absence (100% irrigated - control) of the stress. The cDNAs were sequenced and analyzed using software on the Galaxy platform at Embrapa Informática Agropecuária. To confirm the results obtained by RNAseq some genes were submitted for qPCR analysis. For the qPCR we choose 22 genes with no difference in gene expression, 49 up and 29 down regulated genes respectively in the stressed treatment. All of the them were low copy number and associated in certain level to biological mechanism already described in other plant species. For the up and down regulated genes were selected the ones with the difference was greater than 10 times. The results showed consistent with variables less than two times between the two methods. Some of these genes will be tested in transgenic plant lines. This work will help consolidate the results of RNAseq for the expression of genes related to water stress, evidencing as metabolic pathways related to this process.

Funding acknowledgement: Fapemig

P168

ig2 (indeterminate gametophyte 2), encoding a maize Microtubule-Associated Protein 65-3 (MAP65-3) is required for female gametophyte differentiation

(submitted by Antony Chettoor <achettoor@carnegiescience.edu>)
Full Author List: Chettoor, Antony[1]; Evans, Matt[1]
[1] Carnegie Institution for Science, Department of Plant Biology, Stanford,CA 94305

Microtubules (MTs) are highly conserved structural proteins of the plant cytoskeleton and are essential for various cell functions. The de novo synthesis of MT arrays entails complex interactions between tubulin and targeting/activating regulatory proteins. MT dynamics and interactions with other cellular components are regulated by various proteins that act on MT polymers and tubulin subunits. Microtubule –associated proteins(MAPs), a class of MT binding regulators have been shown to bind, bundle and stabilize MTs. We have identified *ig2 (indeterminate gametophyte 2*), a maize mutant with abnormal female gametophyte development. IG2 is the maize ortholog of Arabidopsis MICROTUBULE-ASSOCIATED PROTEIN65-3/PLEIADE (AtMAP65-3/PLE) that is localized to interdigitating microtubules of the spindle and is essential for cytokinesis. Mutant ,*ig2* embryo sacs exhibit large, vacuolated antipodal cells that fail to proliferate and inefficiently express *PIN1* and *DR5* reporters. Mutant embryo sacs also have extra nuclei in central cells and fewer egg cells and synergids. *ig2* mutant ears produce both miniature and aborted seeds as a consequence of the excess central cell nuclei, indicating that they can function as polar nuclei. The *ig2* reference mutant allele was identified by a combination of map based cloning and Mu insertion site sequencing approaches. We utilized the CRISPR/Cas9 system to generate targeted mutations in the putative ig2 gene. These CRISPR/Cas9 generated mutant alleles cause similar defects as the original mutant allele, in both embryo sacs and seeds. These results uncover a new role for MAP 65-3 in female gametophyte development.

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P169

4D analysis of maize roots reveals how genotype-specific differences in individual root growth patterns leads to distinct architectures

(submitted by Christopher Topp <ctopp@danforthcenter.org>)
Full Author List: Jiang, Ni[1]; Topp, Christopher N[1]
[1] Donald Danforth Plant Science Center; Saint Louis, MO, 63132

Root system architecture (RSA) has a very important role in plant growth and productivity. Understanding how root systems grow and respond to diverse environments will help to improve plant performance and crop production. However, a fundamental question in the study of root systems is how the growth and development of each root, influenced by its local environment, contributes to the architectural patterns and function of the root system as a whole. We have developed an automated optical imaging system that allows three dimensional (3D) monitoring of root growth processes over time (4D). Plants of two maize genotypes, B73 and Mo17, were imaged every 4 hours for a week, until day 11 after germination. Time-series of 3D root shapes were reconstructed using our RSA-Gia pipeline and then analyzed using DynamicRoots software and an R-based Shiny application we developed (ShinyRoots). DynamicRoots allows automatic computation of structural and dynamic traits of each branch in the entire root system. ShinyRoots computes overall root morphological traits, growth rates, root growth directions and branching patterns, and lateral root distributions. Reactive plots allow the user to interactively view the growth process of selected root branches, the dynamics of traits for individual branches during observation time, and the volume, length, and radial distribution of lateral roots by branching order at every observation time, among other traits. We use these data to model root growth using logistic functions, which reveal the underlying differences in root architectures of B73 and Mo17 as a function of aggregate single-root growth patterns. Ultimately, we envision this work will lead to development of empirically-driven, probability-based growth models that can predict root growth and root-environment interactions with high spatiotemporal resolution as a function of genotype.

P170

A genetic screen to identify maize mutants with cell division defects during stomata formation

(submitted by Amanda Wright <amanda.wright@unt.edu>)
Full Author List: Miles, Nicholas W.[1]; Wright, Amanda J.[1]
[1] University of North Texas; 1155 Union Circle; Denton, TX, 75203

Stomata formation in the grasses is an invariant process that requires both symmetric and asymmetric cell divisions. Previous analysis of maize mutants with defects in stomata formation has provided new knowledge about signaling pathways, cytoskeleton organization, preprophase band formation, and additional aspects of cell division. We initiated a screen for new maize mutants with defects in stomatal complex organization due to abnormal cell divisions. We screened 1000 EMS mutagenized F2 families generated by Clinton Whipple and grown by Madelaine Bartlett. We collected leaf tips from up to 20 mature individuals from each family and made glue impressions to view the organization and patterning of the stomatal complexes. While we originally identified 23 families that segregated putative mutants with defects in stomata organization, only 7 of those putative families segregated mutant plants after one round of back or outcrossing. We are in the process of using next generation sequencing to map our mutations to genomic positions while we continue with additional rounds of out and backcrossing.

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P171

A new maize *tassel seed* mutant is under the control of a two-locus system

(submitted by Silvio Salvi <silvio.salvi@unibo.it>)
Full Author List: Salvi, Silvio[1]; Bovina, Riccardo[1]; Emanuelli, Francesco[1]; Zamariola, Linda[1]; Giuliani, Silvia[1]; Frascaroli, Elisabetta[1]; Landi, Pierangelo[1]
[1] Dipsa, University of Bologna, viale Fanin 44, Bologna, Italy I-40127

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 F2 population (genotyped with 15K SNP array) and by SNP-based bulk segregant analysis using two additional populations (BC1 and F2). Both approaches clearly indicated that the tassel seed phenotype is under the control of two loci mapping on chromosomes 2 and 6. A strong and unexplained recombination suppression across chromosome 2 precluded the characterization of the locus on such chromosome. On the contrary, the chromosome 6 locus was mapped to a < 2 Mb region on bin 6.07. Further fine mapping analysis using 2,000 F2 recombinants and corresponding F3 and F4 families enabled us to narrow down the tassel seed 6.07 locus to a 130 kb region which included three genes based on B73 genome annotation. Candidate genes are being further characterized and tested by comparison of allele sequences and gene expression analysis.

P172

A novel meristem regulation mechanism: signaling from primordia to stem cells in maize

(submitted by Fang Xu <fxu@cshl.edu>)
Full Author List: Xu, Fang[1]; Je, Byoung Il[1]; Jackson, David[1]
[1] Cold Spring Harbor Laboratory, NY, 11724

Stem cells are critical to the development of all multicellular organisms. While much is known about communication between the stem cells and supporting niche cells, very little is understood about potential signals from differentiating progeny cells that feed back to the stem cell niche. In plants, the size of the shoot apical meristem (SAM) is regulated by balancing stem cell proliferation with the incorporation of daughter cells into primordia. This balance is maintained by CLAVATA-WUSCHEL feedback signaling between stem cells and underlying niche cells. Studies on *FEA3* (*FASCIATED EAR 3*) in maize, which encodes a novel CLV-related receptor-like protein, led to the discovery of a fundamentally new mechanism in which signals from differentiating cells feedback to the niche. FEA3 is expressed lower down in the SAM than CLV1, and perceives the ZmFCP1 peptide, which is expressed in differentiating cells of primordia, to suppress WUSCHEL expression in the region below the organizing center. To better understand the functional mechanism of FEA3, a *fea3* EMS suppressor screen was carried out to identify potential downstream signaling components. Also, transgenic plants of FEA3 fused with N-terminal or C-terminal tandem affinity purification (TAP) tags (YFP-STREP tag) were constructed, and will be used for tandem affinity purification followed by Mass spectrometry to isolate FEA3 interactors. In addition, CRISPR technology is being used to knock out FEA3 homologs, to study the function of FEA3 like receptor-like proteins in maize, which might be involved in meristem development regulation. Further characterization of the FEA3 pathway could provide new ways to increase seed yield in maize and other crops.

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P173

A significant increase of heterofertilization rate gain insights into in vivo haploid induction in maize

(submitted by Xiaolong Tian <544426657@qq.com>)
Full Author List: Tian, Xiaolong[1]; Chen, Baojian[1]; Qin, Yuanxin[1]; Li, Xingli[1]; Chen, Shaojiang[1]
[1] National Maize Improvement Center of China, China Agricultural University, Yuanmingyuan West Road, Haidian District, Beijing, China, 100193

Chromosome elimilation and single fertilization are two main hypotheses raised to explain the in vivo haploid formation in maize. However, the exact mechanism of haploid induction remains unclear. Herein, we introduce a method to produce heterofertilization kernels(HFK) with a higher rate(>5%) which may gain our insights into the haploid induction in maize. Dual pollination were applied in our research. Both of the type-1 and type-2 HFK rate significantly increases(up to 2.74% and 2.96%, respectively) when inducer CAU5 was used as the first pollinator and the high oil inbred lines GY923 as the second pollinator, which remind us of the fertilization recovery after defective sperm cell release in Arabidopsis. More germplasms, inducers and inbred lines will be tested to verify the universality of our results. Any labs interested in haploid induction, heterofertilization and double fertilization are suggested to try this method.

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P174

Agrobacterium tumefaciens vacuum infiltration of *Zea mays* for transient expression

(submitted by Mary Rath <mary.rath@smail.astate.edu>)
Full Author List: Rath, Mary M.[1]; Moore, Kayla[2]; Booth, Amber[1]; Hood, Elizabeth E.[1]
[1] Arkansas Bioscience Institute; 504 University Loop; Jonesboro, Arkansas, USA 72401
[2] University of Arkansas at Fort Smith; 5210 Grand Ave, Fort Smith, Arkansas, USA 72904

Agrobacterium tumefaciens has been a helpful tool for transient and stable transformation of select species, including *Zea mays*. This study utilizes the vacuum infiltration method standardized for *Nicotiana* spp. to transiently transform corn leaves. Due to the hypervirulent nature of helper strain EHA101, a derivative of A281, gene transfer to corn leaves for transient expression has been made possible. With the consideration of factors such as cell concentration, temperature of incubation, and surface area, a standardized protocol for vacuum infiltration of *Zea mays* leaves has been developed. β-Glucuronidase (GUS) is a ubiquitous reporter gene used in this study to verify the success of the vacuum infiltrations. The GUS assay will help to quantify the amount of expression, including when expression peaks, over the course of seven days post infiltration. Three genotypes have been tested using this method: A and B lines, the parents of the Hi II transformation genotype, and a cross of AxB, or Hi II. In earlier trials of infiltration, whole plants were infiltrated without obvious success. In efforts to increase surface area during infiltration, leaf strips have been the major focus of the study. This method of transiently transforming *Zea mays* should create a gateway to testing gene function in maize prior to moving ahead with stable transformation.

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P175

Analysis of meiosis in maize

(submitted by Joke De Jaeger-Braet <joke.jaeger-braet@uni-hamburg.de>)
Full Author List: De Jaeger-Braet, Joke[1]; Balboni, Martina[1]; Schnittger, Arp[1]
[1] University of Hamburg - Biozentrum Klein Flottbek - Department of Developmental Biology; 22609 Hamburg; Ohnhorststrasse 18; Germany

Meiosis is essential for sexual reproduction as it maintains genome size from one generation to the other. Moreover, meiosis is also the driving force for biodiversity, whereby it is important in plant breeding. Despite of its importance, many crucial steps in meiosis are not well understood, e.g. how the recombination pattern is determined. The analysis of meiosis in crops such as maize is complicated since it is often not obvious which genes are the functional homologs of already well-characterized meiotic regulators in other species. Thus, the identification of meiotic genes is an important process in crop plants. Here, we compare well-characterized meiotic genes from other model systems with what is known in maize as a starting point for subsequent molecular and cytological studies. Particular focus is on gene organization and expression analyses.

P176

Anomalous splicing of U12-type introns underlies the developmental defects of a maize mutant in a novel RNA binding motif protein 48 (*rbm48*)

(submitted by Shailesh Lal <lal@oakland.edu>)
Full Author List: Lal, Shailesh K.[1]; Bai, Fang[2]; Shodja, Donya[1]; Brigolin, Christian J.[1]; Martin, Federico[2]; Tseung, Chi-Wah[2]; Barbazuk, W. Brad[2]; Settles, A. Mark[2]
[1] Department of Biological Sciences, Oakland University, Rochester Hills, MI 48309
[2] Horticultural Sciences Department, University of Florida, Gainesville, FL 32611

Minor U12-type introns reside in the genome of many eukaryotic organisms and are spliced by a distinct spliceosomal machinery. The biological significance of their parallel existence with the major U2-type introns is not well understood. They have been linked to playing regulatory roles in the growth and development in both plants and animals. We identified two mutant alleles in the conserved RNA Binding Motif 48 (*rbm48*) gene. RBM48 protein function has not been defined in any organism. We show that maize *rbm48* mutants have severe defects in seed development with prolonged endosperm cell proliferation and altered cell differentiation similar to the *rough endosperm3* (*rgh3*) U12 splicing mutant. Presence of *rbm48* within eukaryotic genomes correlates with species that retain U12 splicing. RNAseq and RT-PCR analyses revealed aberrant splicing of U12 introns in *rbm48* mutant endosperm. We show that RBM48 localizes within nuclear speckles using transient expression of fluorescence protein fusions. Bimolecular fluorescence complementation and *in vitro* pull down assays show that RBM48 interacts with other splicing factors, including U2 Auxiliary Factor subunits and RGH3. These data indicate that U12 splicing is required for endosperm cells to shift from proliferative growth to differentiate into specialized cell types. Comprehensive annotation of maize minor intron containing genes indicates that cell cycle regulators and production of secreted glycosylated proteins are disrupted in *rbm48* and *rgh3*. These data indicate that U12 splicing plays an important role in cell differentiation.

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P177

Axillary bud development of tillers in domesticated Setaria, its wild ancestor and similarities in domesticated and non-domesticated sorghum, maize and teosinte

(submitted by Muriel Longstaff <mtlongstaff@gmail.com>)
Full Author List: Longstaff, Muriel T[1]; Xiao, YuGuo[1]; Kelly, Jacob A[1]; Ross, Jason[1]; Whipple, Clinton J[1]
[1] Department of Biology, Brigham Young University, Provo,UT USA 84602

Setaria is a panicoid grass related to sorghum and maize. As is common in other grasses, lateral branches called tillers, may form from the base of the central stalk. The domesticated *S. italica* (foxtail millet) and its wild ancestor *S. viridis* (green foxtail) have differences in tiller development and number. Similar to most domesticated cereals, *S. italica* grows few tillers while its wild ancestor grows many. To explore tiller growth development in setaria, we have measured of tiller axillary bud growth from 6 days after planting (DAP) up through 24DAP. Comparative measurements of axillary buds in teosinte, maize B73 inbred, maize mutants *teosinte branched1 (tb1)* and *grassytillers1 (gt1)*, (teosinte and all maize measurements 6DAP-16DAP), *Sorghum verticiliflorum* (measurements from 8DAP-16DAP), and the *Sorghum bicolor* lines BTx623 and Tx7000 (measurements from 8DAP-22DAP). In all wild ancestors, tiller buds initiate and eventually grow into tillers. We have found in the measurements of domesticated maize B73 inbred, the maize mutants, and Sorghum BTx623 and Tx7000, buds are initiated and then go dormant. In *S. italica* however, axillary buds did not always initiate but when they did they occassionally go dormant. To explore this difference in tiller establishment further, scanning electron microscopy (SEM) is underway to understand the early events in tiller initiation in setaria.

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P178

Blue light phototropism in maize: translation from model systems

(submitted by Diana Coats <coatsd@missouri.edu>)
Full Author List: Coats, Diana R[1]; Liscum, Emmanual[1]; McSteen, Paula[1]
[1] University of Missouri; Columbia, Missouri, 65211

Plants utilize several classes of light receptors to perceive environmental light cues. These cues are used to mediate adaptive growth changes to maximize photosynthetic light capture and plant success. One specific class of photoreceptors, the phototropins, activate the blue light signaling pathway necessary for responses such as phototropism, stomatal opening, cotyledon and leaf expansion, and root architecture. Phototropism, a growth response to directional light cues, has been used to observe plant movement responses even before Darwin's The Power of Movement in Plants. While various crop seedlings, including maize, were popular models for many of the classical physiological studies, the molecular mechanisms underlying phototropism, and blue light adaptive responses have recently been more extensively studied in model organisms such as Arabidopsis. These studies have elucidated many components of the molecular mechanism underlying blue light perception, signaling and response. We aim to determine the signaling components involved in blue light perception and response in maize, with the ultimate goal of understanding their role in developmental changes in response to stress, specifically drought. We are currently utilizing a forward and reverse genetic approach, followed by a classic phototropism assay, to identify putative components of the tropic response pathway in maize.

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P179

Brassinosteroids control inflorescence development in panicoid grasses

(submitted by Jiani Yang <jyang@danforthcenter.org>)
Full Author List: Yang, Jiani[1]; Thames, Shuiyi[1]; Jiang, Hui[1]; Huang, Pu[1]; Best, Norman B.[2]; Dilkes, Brian P.[2]; Eveland, Andrea L.[2]
[1] Donald Danforth Plant Science Center, Saint Louis, MO 63132, USA
[2] Department of Biochemistry, Purdue University, West Lafayette, Indiana 47907, USA

Brassinosteroids (BRs) are phytohormones implicated in developmental control of plant architecture, including inflorescence architecture, an important agronomic trait in cereal crops. In maize, BR biosynthesis mutants (e.g. *na1, na2, brd1*) are dwarf and develop feminized tassels. Despite prominent phenotypes, little is known about the mechanisms by which BRs modulate panicle development. Here, using *Setaria viridis* as a model system, we cloned the *bristleless1* (*bsl1*) gene, which encodes a cytochrome P450 involved in BR biosynthesis; the syntenic ortholog of *Dwarf11* (*D11*) in rice. The *bsl1* mutant was identified because it exhibits severe reduction of bristles in its inflorescences. Bristles are modified, sterile branches unique to the "bristle clade" of grass species, and are produced along with spikelets in the *Setaria* inflorescence, apparently in place of paired spikelets characteristic of maize and sorghum. Our analysis of the *bsl1* mutant suggests that BRs act in the spikelet meristem fate decision to differentiate into a bristle or spikelet. In contrast to normal *Setaria* flowers, lower florets of *bsl1* spikelets do not abort and appear to develop normally, reminiscent of maize sex-determination mutants, such as *tasselseed2*. *In situ hybridization* in inflorescence primordia showed that *bsl1* transcripts localized to the adaxial side of developing primary branches and accumulated at lateral organ initiation sites in spikelet meristems, suggesting a function for *bsl1* in both meristem fate and lateral organ initiation. We further showed that the maize ortholog, *ZmD11*, was expressed in analogous patterns in ear and tassel primordia, suggesting a conserved role in maize. RNA-seq data also support a role for BRs in regulating inflorescence development through modulation of known regulators. The *bsl1* mutant therefore provides an ideal system for studying BRs in regulation of panicoid inflorescence development and their role in maize sex-determination.

P180

Cereal roots enact austerity measures during drought to bank water

(submitted by Jose Sebastian <jsebastian@carnegiescience.edu>)
Full Author List: Sebastian, Jose[1]; Yee, Muh-Ching[1 5]; Viana, Willian Goudinho[2]; Priest, Henry[3]; Hochholdinger, Frank[4]; Brutnell, Thomas[2]; Dinneny, Jose[1]
[1] Carnegie Institution for Science, Department of Plant Biology, Stanford, CA, 94305
[2] CAPES Foundation, Ministry of Education of Brazil, Brasilia, DF, Zip Code 70.040-020
[3] Danforth Plant Science Center, St. Louis, MO
[4] University of Bonn, INRES - Crop Functional Genomics, Friedrich-Ebert-Allee 14453113
[5] Stanford University, Stanford CA 94305

Many important cereal crops such as rice, wheat are members of the Poaceae family, which develop root systems characterized by a high-degree of root initiation from the belowground basal nodes of the shoot, termed the crown. While this post-embryonic shoot-borne root system (crown roots) represents the major conduit for water uptake, little is known regarding what effect water availability has on its development. Our data demonstrate that in the newly developed cereal crop model plant Setaria viridis, the crown locally senses water availability and suppresses post-emergence crown root growth under water deficit. This response was observed in field and growth room environments and in all grass species tested. Luminescence-based imaging of root systems grown in soil revealed a shift in root growth from crown to primary-root derived branches, suggesting that primary-root-dominated architecture can be induced in S. viridis under certain stress conditions. Crown roots of Zea mays (maize) and Setaria italica, domesticated relatives of teosinte and S. viridis, respectively, show reduced sensitivity to water deficit, suggesting that this response may have been influenced by human selection. Enhanced water status of maize mutants lacking crown roots suggests that, under water deficit, stronger suppression of crown roots may actually benefit crop productivity. Currently, several approaches including forward genetics screens are employed to explore the underlying molecular machinery regulating crown root response to water deficit.

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P181

Characterization and cloning of a temperature sensitive maize mutant affecting inflorescence development

(submitted by Qiujie Liu <qiujieliu@waksman.rutgers.edu>)
Full Author List: liu, qiujie[1]; Federici, Silvia[1]; Galli, Mary[1]; Gallavotti, Andrea[1]
[1] Waksman Institute of Microbiology , 190 Frelinghuysen Road Piscataway, New Jersey 08854-8020

Meristems are groups of undifferentiated stem cells in plants. Apical meristems establish the main axis of plant growth while axillary meristems, formed at the axils of true or modified leaves, are responsible for lateral branch and flower formation, which are essential for maize reproduction. *bif173** is a single recessive *barren inflorescence (bif)* mutant generated by EMS mutagenesis. Similar to other *bif* mutants, *bif173** mutants develop tassels with fewer branches and spikelets, and ears with unorganized kernels and partially barren tips. SEM analysis and in situ hybridizations suggest that axillary meristem initiation and development are defective in *bif173** mutants. Interestingly, *bif173** mutants show a more severe phenotype at higher temperatures, suggesting that *bif173** is a temperature sensitive mutant. Moreover, we discovered a strong genetic interaction between *bif173** and the auxin signaling mutant *Bif1*, which suggests that *BIF173** may influence auxin biology. To identify the gene responsible for the *bif173** mutation, an initial Bulk Segregant Analysis (BSA) and fine mapping were performed and positioned *BIF173** within a 1.2 Mb window on chromosome 8. A subsequent RNA-seq approach uncovered a candidate gene with a mutation in a conserved amino acid. Since only one *bif173** mutant allele is currently available, we are following several complementary approaches to confirm the candidate gene. We developed maize transgenic lines expressing the candidate gene from its native promoter to test whether it can rescue the mutant phenotype. At the same time, we are employing a *CRISPR/Cas9* based approach to generate new lesions in our candidate gene. Furthermore, we generated mutations in the *Arabidopsis* homologous genes for a heterologous complementation approach. Surprisingly, the candidate gene encodes a mitochondria-localized protein. If confirmed, our research should help us to understand the role of a mitochondria-localized protein in inflorescence development and its influence on auxin biology.

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P182

Characterization and mapping of the suppressor of sessile spikelet 3 (Sos3) mutant which functions in paired spikelet development in maize

(submitted by Amanda Blythe <amb4x2@mail.missouri.edu>)
Full Author List: Blythe, Amanda[1]; Adkins-Threats, Mahliyah[2]; Wooten, Shelbie[1]; McSteen, Paula[1]
[1] University of Missouri; Department of Biological Sciences; Columbia, MO, 65201
[2] Truman State University; Kirksville, MO 63501

Zea mays (maize) and rice are two of the most important grasses in the world due to their central roles in agriculture. The spikelet, a short branch which produces florets, is the fundamental unit of grass inflorescences. This structure is important because the florets ultimately give rise to the grains of the plant. However, the key difference between these grasses is the number of spikelets produced. In particular, maize produces paired spikelets while rice and wheat produce single spikelets. In order to study this trait, the *Suppressor of sessile spikelet 3* (*Sos3*) mutant of maize is being analyzed. *Sos3* produces single instead of paired spikelets, causing defects in the development of the male (tassel) and female (ear) inflorescences. The resulting phenotype is characterized by fewer tassel branches and gaps between kernels on the ears. For these reasons, the *sos3* gene may play a role in the evolution of the paired spikelet. Characterization of mutant phenotypes through histology and scanning electron microscopy (SEM) shows that single spikelets are produced in place of paired spikelets, thus indicating that the *sos3* gene functions early in plant development. To determine the location and identity of the mutated gene, the *Sos3* mutant is being mapped. Linkage analysis with microsatellite markers shows *Sos3* maps to chromosome 1 (bin 6) between markers umc1988 and umc2025, and fine mapping is continuing. Identifying the gene responsible for the *Sos3* mutation will provide valuable insight into spikelet development not only in maize but also in other cereals, such as rice. Applying this knowledge to single spikelet species could potentially lead to increased yields, thus advancing the field of agriculture.

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P183

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

(submitted by Edgar Demesa-Arevalo <edemesaa@cshl.edu>)
Full Author List: Demesa-Arevalo, Edgar[1]; DeBlasio, Stacy[2]; Claeys, Hannes[1]; Skopelitis, Tara[1]; Satoh-Nagasawa, Namiko[3]; Char, Si Nian[4]; Yang, Bing[4]; Jackson, David[1]
[1] Cold Spring Harbor Laboratory, 1 Bungtown Road, Cold Spring Harbor, NY11724, USA
[2] Boyce Thompson Institute for Plant Research, Ithaca NY14853, USA
[3] Laboratory of Plant Genetics and Breeding, Akita Prefectural University, Akita 010-0195, Japan
[4] Department of Genetics, Development and Cell Biology, Iowa State University, Ames, IA 50011, USA

The classical maize mutant *ramosa3* (*ra3*) shows increased branching in inflorescences; RA3 encodes a trehalose phosphate phosphatase enzyme, expressed at the base of inflorescence axillary meristems. However, RA3 protein is localized in nuclear and cytoplasmic speckles, 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 ATACHMENT FACTOR B (*Zm*SAFB) and *Zea mays* VASCULAR PLANT ONE–ZINC FINGER (*Zm*VOZ). SAFB has been described in animal system as a RNA Recognition motif (RRM) protein involved in transcriptional co-repression and splicing, but its functions in plants are not described 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 are been analyzed for enhancement of the *ra3* phenotype in double or multiple mutant combinations. Additionally, *Zm*SAFB-mRFP1 transgenic lines showed constitutive expression in a punctate pattern in nuclear compartments, and partial co-localization with the active form of RNA POLII, suggesting a role in transcriptional regulation. *Zm*VOZ1-RFP lines have also been generated, and YFP-*Zm*VOZ5 is under construction. 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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P184

Characterization of the *ba*CL* mutant in maize

(submitted by Taylor Smith <tmshd4@mail.missouri.edu>)
Full Author List: Smith, Taylor[1]; Yao, Hong[1]; Lunde, China[2]; McSteen, Paula[1]
[1] University of Missouri, 1200 Rollins St, Columbia, MO 65211
[2] Plant Gene Expression Center, 800 Buchanan St, Albany, CA 94710

Maize produces two types of reproductive structures - the tassel and the ear. The tassel consists of a main spike with several long branches and the ear shoot develops from the main stalk. Both the tassel and ear are crucial for crop yield; hence studying their development is important in agriculture. Several classes of mutants affect tassel and ear development. The *barren inflorescence* class of mutants is defective in tassel and ear development often due to defects in the plant growth hormone auxin. The *barren stalk* (*ba*) class of mutants is characterized by having no ear shoot and in some cases the tassel is also affected.
A new *barren mutant, ba*CL* is presented that has been found to map to chromosome 6, between bin 6.04 and 6.05, and represents a new locus. SEM analysis shows that the immature tassels of this mutant have defects that differ from either *ba* or *bif* mutants. Histology of early plant development shows the mutant does not make an ear at all, rather than making an ear early on and then terminating. When crossed to *teosinte branched 1* (*tb1*), the double mutants produced fewer tillers than *tb1* indicating that there are defects in tiller development also. Phenotypic analysis shows defects in leaf phyllotaxy and the formation of leaf flaps. Further characterization of the mutant is being performed to understand its role in development and fine mapping is being carried out in order to clone the gene.

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P185

Characterization of the mitosis-to-meiosis transition in pollen mother cells by EdU incorporation

(submitted by Ching-Chih Tseng <tsengyin@gate.sinica.edu.tw>)
Full Author List: Tseng, Ching-Chih[1 2]; Shi, Yun-Zhi[1]; Wang, Chi-Ting[1]; Kao, Yu-Hsin[1]; Wang, Chung-Ju Rachel[1]
[1] Institute of Plant and Microbial Biology, Academia Sinica, Taipei, 11529, Taiwan
[2] Institute of Plant Biology, National Taiwan University, Taipei, 10617, Taiwan

In flowering plants, pollen mother cells (PMCs) are first proliferated by mitosis, and then enter meiosis to produce male haploid gametes. How the mitosis-to-meiosis transition works is unknown in plants. I monitor this transition by labeling DNA replication in maize anthers. Results revealed that differentiated male germ cells first undergo asynchronous mitosis in an anther, and the mitotic rate gradually decreases until a cell cycle resting stage in all PMC cells. Next, the pre-meiotic S is initiated synchronously, followed by the prophase I. The *multiple archesporial cells1* (*mac1*) mutant showed that the G1 pause is absent and the continuous mitotic cell divisions result in extra PMCs. Surprisingly, without the G pause, the pre-meiotic S still occurs in some of PMCs; whereas other PMCs are arrested during interphase. The *ameiotic1* (*am1*) mutant fails to enter pre-meiotic S phase. After a prolonged resting stage in am1 PMCs, asynchronous mitosis resumes. The double mutant of *mac1* and *am1* showed additive phenotype of *mac1* and *am1*. Taken together, these results indicate that MAC1 and AM1 are required for the G1 pause and pre-meiotic S phase, respectively, and both genes function independently during this transition.

Funding acknowledgement: Academia Sinica, Taiwan

P186

Characterizing the diversity of brace root architecture and anatomy in maize

(submitted by Erin Sparks <erin.sparks@duke.edu>)
Full Author List: Sapp, Justin T.[1]; Sparks, Erin E.[1]
[1] Duke University; Durham, NC, 27708

Maize brace roots, which emerge from plant stems above the soil, are proposed to play an important role in structural stability and late-stage nutrient/water acquisition. Yet how brace roots develop, integrate environmental cues and contribute to whole plant physiology remains a poorly understood area of plant biology. To quantify the developmental diversity of brace roots, brace root initiation, anatomy and architecture were assayed in a diverse germplasm of inbred maize varieties. A histological atlas of brace root primordia morphology and anatomy was generated to characterize the differences and similarities in brace root development. In addition, field-based above- and below-ground root phenotyping data was obtained from the same germplasm. These results show that there is vast diversity in brace root structure at the level of initiation, anatomy and architecture. To determine the molecular networks that regulate brace root initiation and emergence, the role of auxin in these processes was analyzed. Analysis of a maize mutant that is defective in auxin transport demonstrated a defect in the number of stem nodes that produce brace roots. These results suggest that auxin plays a key role in determining the extent of brace root production in maize. These experiments are among the first to define the diversity of brace root architecture and anatomy in maize, which is critical to understand the functional significance of these specialized roots.

P187

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

(submitted by Lei Liu <lliu@cshl.edu>)
Full Author List: Liu, Lei[1]; Arevalo, Edgar Demesa[1]; Skopelitis, Tara[1]; Je, Byoung Il[1]; Wu, Qingyu[1]; Jackson, David[1]
[1] Cold Spring Harbor Laboratory; 1 Bungtown Road; Cold Spring Harbor, NY USA 11724

The stem cell development of plant meristems is maintained by CLAVATA–WUSCHEL feedback signaling between the stem cell zone at the tip of the meristem and the underlying organizing center. The *CLE* (*CLAVATA3/ EMBRYO SURROUNDING REGION*) genes encode a major group of secreted peptides that function as signals in cell-cell communication, cell proliferation and differentiation. One example, CLV3, is secreted from stem cells at the tip of the meristem and perceived by leucine-rich-repeat (LRR) receptors, such as CLV1, to repress WUS expression. However, most of the CLEs in maize have not been studied, only ZmFCP1 was characterized to be perceived by FASCIATED EAR3 (FEA3). To survey the function of maize CLEs, especially their function on meristem development, we collected all the predicted maize CLE sequences from the literature, which suggested there are 49 predicted CLEs in maize. Then we integrated the expression data of multiple maize tissues, including shoot apical meristem, ear and tassel primodia, immature kernels, leaves, roots and so on, to figure out their expression patterns. We focused on CLEs that show higher expression in SAM, ear and tassel, and are employing the genomic editing technology CRISPR/Cas9 to create mutations of these genes. We are planning to CRISPR 31 CLEs in maize, and one of them, CLE7, for which we already got CRISPR mutants, has a fasciated ear phenotype. We will further CRISPR the promoters of CLEs that show strong influence in ear development, to create weak alleles for enhancement of maize yield production.

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P188

Comparative genetics of ligule development in barley

(submitted by Ron Okagaki <okaga002@umn.edu>)
Full Author List: Okagaki, Ron J[1]; Muehlbauer, Gary J[1 2]
[1] Dept of Agronomy, Univ of Minnesota, St Paul, MN, 55108, USA
[2] Dept of Plant Biology, Univ of Minnesota, St Paul, MN, 55108, USA

The grass leaf blade - sheath boundary is characterized by the ligule and auricle, and the maize ligule is a model system for studying plant development. We are comparing barley ligule development with maize. Here we present results on three genes controlling barley ligule formation. The barley liguleless1 (lig1) gene is orthologous to maize lg1. Similar to maize lg1, the barley lig1 mutants completely lack ligules and auricles. Uniculme4 (cul4) is homologous with the maize Tassels replace upper ear1 and Arabidopsis Blade-on-petiole genes; cul4 mutant plants lack ligules but have normal auricles. Eligulum-a (Eli-a) encodes a previously unknown plant protein; auricles are reduced in size and ligules may be completely absent. Results from double mutant analysis and RNA in situ hybridizations indicated that LIG1 is expressed early where it helps define the blade-sheath boundary and controls ELI-A and CUL4 expression. This model is based on the following observations: 1) LIG1 and ELI-A transcripts are detected in leaf primordia before CUL4 transcripts; 2) LIG1 transcript is present in eli-a mutants but ELI-A transcript is not found in lig1 mutants; and 3) ligules and auricles in the eli-a.18; cul4 double mutant are very similar to lig1. The roles of these genes in axillary meristem development will also be discussed.

P189

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

(submitted by Angel Del Valle Echevarria <angeldve@hawaii.edu>)
Full Author List: Del Valle-Echevarria, Angel R.[1]; Uyehara, Aimee N.[1]; Cahill, James F.[1 2]; Nelissen, Hilde[3]; Hunter, Charles[4]; Jander, Georg[5]; Muszynski, Michael G.[1 2]
[1] Dept. of Tropical Plant and Soil Sciences, University of Hawai‘i at Mānoa, Honolulu, HI, USA 96822
[2] Dept. of Genetics, Development and Cell Biology, Iowa State University, Ames, IA, USA 50011
[3] Department of Plant Systems Biology, VIB, B-9052 Gent, Belgium
[4] Chemistry Research Unit, CMAVE-USDA, Gainesville, FL, USA 32608
[5] Boyce Thompson Institute, Ithaca, NY, USA 14853

Plant growth is mediated by two cellular processes: division and elongation. The maize leaf is an excellent model to study plant growth since these processes are spatially separated into discreet zones - a division zone (DZ), transition zone (TZ), and elongation zone (EZ) - at the base of the leaf. We are studying a semi-dominant maize mutant named *Hairy Sheath Frayed1 (Hsf1)* that displays reduced leaf growth caused by cytokinin hypersignaling. Cytokinin (CK) is a well-studied hormone which typically functions to promote cell proliferation but, depending on cellular context, it can also repress growth; although how repression is mediated is not well-defined. During our analysis of *Hsf1*, we discovered that the mutant over accumulates jasmonic acid (JA), a hormone previously shown to repress cell division and growth. This result suggested CK may crosstalk with JA in the control of leaf growth, which is a previously unrecognized connection and may explain one route by which CK can repress growth in certain tissues. We evaluated JA pathway gene expression levels in the division zone and elongation zones of the emerging leaf #4 of *Hsf1*/+ and wild type (WT) sibs by qRT-PCR. Several JA biosynthesis genes were significantly upregulated in the growth zone of mutants compared to WT sibs. In parallel, we used a bioinformatics approach to identify candidate transcription factors associated in gene regulatory networks (GRNs) with JA pathway genes. Based on this survey, we identified a transcription factor that was also CK responsive, as its expression level in the *Hsf1* leaf growth zone was also significantly upregulated. Additional molecular and genetic studies will be presented suggesting that this proposed interaction contributes to leaf growth control.

P190

Dissecting the genetic basis for meristem size control and branch initiation during grass inflorescence development using Setaria viridis as a model

(submitted by Chuanmei Zhu <czhu@danforthcenter.org>)
Full Author List: Zhu, Chuanmei[1]; Box, Mathew[1]; Goad, David[1]; Kellogg, Elizabeth[1]
[1] Donald Danforth Plant Science Center, 975 N Warson Rd, St. Louis, MO 63132

Inflorescence architecture at maturity directly impacts crop yield. Characteristics of the inflorescence, such as order of branches, pairing and number of spikelets can be reflected at early developmental stages. However, much remains to be discovered about the genetic controls of early inflorescence development. In this project, we are using Setaria viridis as a model to understand the genetic control of meristem maintenance and branch initiation, which are crucial to understand the mechanism of early inflorescence development. For this, we studied the evolution of CLE genes and are testing their function using in situ and CRISPR-CAS techniques in Setaria. CLEs are CLV3-like genes whose function in meristem maintainance has been examined in Arabidopsis but their function in grass inflorescence development remains poorly understood. In addition, we are investigating the role of a family of auxin importer proteins in early inflorescence development. While function of auxin efflux carriers such as PIN1 have been extensively characterized during inflorescence development, the role of auxin influx carriers remains unstudied. We have shown that mutation in one of the auxin importer genes in Setaria results in initiation of fewer branches and thus fewer spikelets, suggesting a critical role of auxin importer genes in grass inflorescence development. RNAseq analysis and various imaging techniques have been employed to understand the functional mechanism of auxin importer genes. Together, these studies will provide insights in understanding the molecular mechanisms of meristem control and branch formation during grass inflorescence development.

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P191

E+ subgroup PPR protein Defective Kernel 36 is required for multiple mitochondrial transcripts editing and seed development in maize and *Arabidopsis*

(submitted by Guifeng Wang <holdonhero2000@shu.edu.cn>)
Full Author List: Wang, Gang[1]; Zhong, Mingyu[1]; Shuai, Bilian[1]; Song, Jiandong[1]; Zhang, Jie[1]; Han, Liang[1]; Ling, Huiling[1]; Tang, Yuanping[1]; Wang, Guifeng[1]; Song, Rentao[1 2]
[1] Shanghai Key Laboratory of Bio-Energy Crops, School of Life Sciences, Shanghai University, Shanghai 200444, China
[2] National Maize Improvement Center of China, China Agricultural University, Beijing 100193, China

Mitochondria are semi-autonomous organelles that are the powerhouse of the cells. Plant mitochondrial RNA editing guided by pentatricopeptide repeat (PPR) proteins is essential for energy production. We identify a maize defective kernel mutant *dek36*, which produces small and collapsed kernels, leading to embryos and/or seedlings lethality. Seed filling in *dek36* is drastically impaired, in line with the defects observed in the organization of endosperm transfer tissue. Positional cloning reveals that *DEK36*, encoding a mitochondria-targeted E+ subgroup PPR protein, is required for mitochondrial RNA editing at *atp4-59*, *nad7-383* and *ccmFN-302*, thus resulting in decreased activities of mitochondrial complex I, complex III and complex IV in *dek36*. Loss-of-function of its *Arabidopsis* ortholog At DEK36 causes arrested embryo and endosperm development, leading to embryos lethality. *At_dek36* also has RNA editing defects in *atp4*, *nad7*, *ccmFN1* and *ccmFN2*, but at the non-conserved sites. Importantly, efficiency of all editing sites in *ccmFN1*, *ccmFN2* and *rps12* is severely decreased in *At_dek36*, probably caused by the impairment of their RNA stabilization. These results suggest that the DEK36 ortholog pair are essential for embryo and endosperm development in both maize and *Arabidopsis*, but through divergent function in regulating RNA metabolism of their mitochondrial targets.

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P192

Essential role of biotin in embryogenesis of maize and rice

(submitted by Masaharu Suzuki <masaharu@ufl.edu>)
Full Author List: Suzuki, Masaharu[1]; Sugiki, Ai[2]; Wu, Shan[1]; Hanson, Andrew D.[1]; McCarty, Donald R.[1]; Sato, Yutaka[3]
[1] PMCB program, Horticultural Sciences Dept., University of Florida, Gainesville, FL32611
[2] Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya, Aichi 464–8601, Japan
[3] Plant Genetics Lab., National Institute of Genetics, Mishima, Shizuoka 411-8540, JAPAN

Genetic analyses of embryo defective mutants have revealed numerous genes required for embryogenesis in Arabidopsis. Surprisingly, only a limited number of the genes essential for embryogenesis thus far have been identified in cereals. To aim at identification of genes regulating organ differentiation of cereal embryo, we screened *globular embryo* mutants (*gle*) from a large collection of embryo lethal mutant stocks of maize and rice. In *gle* mutants, the embryos arrest organ differentiation at globular stage without affecting endosperm development. Thus, *GLE* genes are required specifically for early organogenesis of embryo. Among rice and maize *gle* mutants, we have identified mutations in an orthologous *GLE* gene, which is required for biotin biosynthesis. Biotin is a water-soluble vitamin that functions as an enzyme cofactor belonging to the prosthetic group. Biotin conjugated enzymes catalyze reactions of carboxylation, decarboxylation and transcarboxylation in important metabolic pathways such as carbohydrate and lipid metabolism. We have obtained two alleles of *gle* mutant in maize. Whereas a null allele of the *gle* mutant developed early defective embryo similar to rice orthologous *gle* mutant, a hypomorphic allele of the mutant was capable to develop normal seeds. With rice *gle* mutant, we confirmed that biotin contents in the mutant seeds are markedly reduced. In addition, exogenously applied biotin was capable to rescue developing seeds of the mutant. These results indicated that lack of sufficient biotin causes arrest of embryo differentiation at globular stage in the *gle* mutant. Analysis of gene expression by using tissue and cell specific markers suggested that *gle* mutant embryo fails to establish proper organization of protodermal cells. These results provide insight into the role of biotin in organogenesis of embryo in cereal grains.

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P193

Exploring the connections between SCARECROW and auxin in maize development

(submitted by Janlo Robil <jmrobil@mail.mizzou.edu>)
Full Author List: Robil, Janlo M[1]; McSteen, Paula[1]
[1] Division of Biological Sciences, University of Missouri, Columbia MO, 65211

The discovery of the key role of SCARECROW (SCR) in establishing Kranz anatomy in the maize leaf by regulation of bundle sheath (BS) identity opened up new directions not only for C4 photosynthesis research but also for plant developmental biology. Although the regulatory network involving transcriptional regulation of SCR has yet to be unraveled, the connection between this transcription factor and the plant growth hormone auxin has become apparent based on genetic and histological evidence. In addition to abnormal differentiation and proliferation of BS cells, *scarecrow* mutants also exhibit fused and misaligned veins and a reduction of vein density in leaves and defects in tassel morphology. These tassel and vein defects are also seen in auxin transport mutants and in plants treated with auxin transport inhibitors. Moreover, crosses between *scr* and auxin transport and biosynthesis mutants exhibited enhanced defects in leaves and tassels. Here, we present histological and genetic evidence supporting possible crosstalk between SCR and auxin. We highlight defects in both vegetative and reproductive organs at different stages of development and characterize patterns of auxin transport using the ZmPIN1 fluorescent marker.

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P194

Expression analysis of maize heterotrimeric Gγ subunits

(submitted by Jara Oppenheimer <oppenheimerjara@gmail.com>)
Full Author List: Oppenheimer, Jara[1]; Deke, Jennifer[1]; Stateczny, Dave[1]; Kluth, Jantjeline[1]; Bommert, Peter[1]
[1] Department of Developmental Biology, University of Hamburg, Germany

Heterotrimeric G protein signaling is known to facilitate the transduction of extracellular signals in animals and plants. 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 manifested in the pleiotropic phenotype of Gα 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 have an additional cysteine rich C-terminal region and depending on which subunit may have a prenylation motif.
As part of a functional genomics approach to elucidate the function of maize Gγ subunits, we are aiming to characterize the tissue specific and subcellular expression pattern of all six maize Gγ subunits, representing one Type A, one Type B and four Type C γ subunits.
We will present initial localization data, based on the analysis of both, C- and N-terminally YFP-tagged cDNA constructs. Those constructs have been either transiently expressed in maize leaves by particle bombardment or in tobacco leaves via agrobacterium-mediated infiltration. We are also engineering stably transformed fluorescent reporter lines of maize Gγ subunits in its genomic context.

Funding acknowledgement: German Research Foundation (DFG)

P195

Expression profile comparison of autonomous temperate maize and photoperiod-dependent Teosinte reveals both distinct and common components that control flowering

(submitted by Mark Minow <mminow@uoguelph.ca>)
Full Author List: Minow, Mark A.A.[1]; Avila, Luis[2]; Turner, Katie[1]; Ponzoni, Elena[3]; Mascheretti, Iride[3]; Lukens, Lewis[4]; Rossi, Vincenzo[3]; Colasanti, Joseph[1]
[1] Dept. of Molecular and Cellular Biology, University of Guelph, Guelph, Ontario, Canada
[2] Dept. of Plant Sciences, University of California, Davis, California
[3] Unità di Ricerca per la Maiscoltura – CREA, Bergamo, Italy
[4] Dept. of Plant Agriculture, University of Guelph, Guelph, Ontario, Canada

Temperate maize (*Zea mays* ssp. *mays*) is essentially a day-neutral, autonomously flowering plant derived from an obligate, photoperiod-dependent tropical ancestor, teosinte (*Zea mays* ssp. *parviglumis*). Flowering in teosinte requires short day (SD) photoperiods (i.e., long nights), such that a brief exposure to light (a night break; NB) will inhibit flowering. Loss of *indeterminate1* (*id1*) gene function severely disrupts the floral transition in temperate maize, causing extreme late flowering. ID1 protein acts exclusively in developing leaves, where it coordinates unknown long distance autonomous signals to induce flowering. ID1 is required for normal carbon partitioning in the maize leaf and loss of *id1* function in the developing leaf affects the mature leaf transcriptome. The link between leaf development and flowering is poorly characterized. We are using mRNA and small RNA genome-wide profiling to examine this process in maize and teosinte. Comparison of flowering (*Id1+*) vs non-flowering (*id1-*) B73 with flowering (SD) vs non-flowering (NB) teosinte revealed only a few common differentially expressed genes during leaf development. Several of these genes are implicated in cell wall remodelling in immature leaves. Known florigen genes displayed altered accumulation in SD/NB teosinte leaves, consistent with the current paradigm for photoperiod-induced flowering. Only one annotated miRNA, *miR399*, had a similar differential expression profile in both B73 and teosinte leaves, suggesting that phosphate sensing may be a shared factor with respect to the floral transition. Although many differentially accumulating small interfering RNA (siRNA) clusters were detected, none were commonly regulated by floral induction in B73 and teosinte. Taken together, these findings support the notion that, in temperate maize, ID1 acts on a pathway largely distinct from the photoperiodic flowering regulation of the wild progenitor teosinte.

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P196

FASCIATED EAR2 perceives different CLE peptides and transmits signals to different downstream components

(submitted by Byoung Il Je <bije@cshl.edu>)
Full Author List: Je, Byoung Il[1]; Wu, Qingyu[1]; Demesa Arevalo, Edgar[1]; Xu, Fang[1]; Meeley, Robert[2]; Jackson, David[1]
[1] Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724
[2] Agricultural Biotechnology, DuPont Pioneer, Johnston, IA 50131

Shoot apical meristems are stem cell niches that balance proliferation with the incorporation of daughter cells into organ primordia. This balance is maintained by feedback signaling between the stem cells at the tip of the meristem, and the underlying organizing center. Recently, we identified FASCIATED EAR3 (FEA3) encoding a leucine-rich repeat receptor-like protein in maize. FEA3 functions in regulation of stem cell proliferation by responding to Zea mays (Zm) FON2-like CLE protein 1 (ZmFCP1) expressed in organ primordia.
Here we show that FEA2 (maize ortholog of CLAVATA2) also participates in ZmFCP1 signaling, but COMPACT PLANT2 (CT2), which is an interactor of FEA2 and encodes the maize G protein alpha subunit, does not. CORYNE (CRN, a membrane localized kinase) is a CLV2 interactor first identified in Arabidopsis. We identified *Zmcrn* mutants showing a fasciated ear phenotype and found that they show resistance to ZmFCP1 peptide treatment, and also confirmed the molecular interaction between FEA2 and ZmCRN. In contrast, ZmCRN and CT2 do not interact physically, and genetic evidence indicates ZmCRN acts in parallel to CT2. These data suggest that FEA2 is involved in perception of diverse peptides and transmits signals differentially to downstream components. This hypothesis shows a new paradigm in signaling pathways of receptor-like proteins.

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P197

Fiesta (Fasciated Internode Erratic Sterile Angustifolia), a new maize mutant to celebrate

(submitted by Sarah Hake <hake@berkeley.edu>)
Full Author List: Singh, Renee[1]; Lunde, China[1]; Lhamo, Dhondup[1]; Pierroz, Grady[1]; Hake, Sarah[1]
[1] Plant Gene Expression Center, 800 Buchanan St. Albany CA 94710

We identified a new recessive EMS mutant with multiple defects in shoot patterning; ears are fasciated, leaves are narrow, and plants are shorter due to erratic internode elongation. Mutants are both male and female sterile. Mutant anthers lack tapetum and carpels form multiple unreceptive silks. These phenotypes are consistent in multiple inbreds. We mapped the locus to an interval on the long arm of chromosome 1 and carried out an RNAseq experiment to identify the mutated gene. We found one likely candidate and ordered an insertion line from the Uniform Mu population. The single homozygote also has a sterile tassel, is shorter and has narrow leaves. Complementation crosses are being evaluated. Future experiments are aimed to determine why defects in this ANGUSTIFOLIA3 ortholog cause such a pleiotropic phenotype.

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P198

Functional analysis of pollen-specific RALFs during reproduction in maize

(submitted by Lele wang <lele.wang@ur.de>)
Full Author List: Wang, Lele[1]; Zhou, Liangzi[1]; Dresselhaus, Thomas[1]
[1] University of Regensburg; Universitätsstrasse 31; Regensburg; Bavaria; Germany; 93053

Small secreted peptides can be classified into two major groups, CRPs (cysteine-rich peptides) and non-CRPs. Previous studies showed that multiple CRPs were 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. Functional studies of RALFs in *Arabidopsis thaliana* revealed that peptides of this gene family were involved in multiple aspects of plant growth and development. For example, it has been shown that RALF interacts with FERONIA in Arabidopsis root development. Based on sequence alignment and expression pattern comparison, several putative FERONIA homologs were found in maize pollen tube with a pollen-specific expression manner. 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. This observation indicated that pollen tubes might be more sensitive when expression levels of *RALFs* were downregulated. The effect of pollen cell wall instability and its consequence is now investigated *in vivo*.

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P199

Genetic interactions between JA and GA pathway genes and inflorescence branching in maize

(submitted by Erik Vollbrecht <vollbrec@iastate.edu>)
Full Author List: Vollbrecht, Erik[1]; Wimalanathan, Kokulapalan[1]; Strable, Joshua[1]; Unger-Wallace, Erica[1]
[1] Department of Genetics, Development and Cell Biology; Iowa State University; Ames, IA, USA, 50011

Suppressor–enhancer screens using the chemical mutagen EMS have been powerful tools in developmental genetic dissection of many processes. For example, this approach has previously identified synergistic or epistatic interactions between genes known by mutant phenotype to regulate inflorescence branching, including the well-studied *ramosa1* (*ra1*), *ra2* and *ra3* genes themselves, as well as their interactors *rel2*, *fea4*, *baf1* and others. Through ongoing suppressor-enhancer screens of *ramosa* mutants we have used map-based and candidate gene approaches to clone several loci that encode genes with direct or indirect roles in metabolism of hormones including JA (*silkless1*, *tasselseed1*, *tasselseed2* and *Tasselseed6*) and GA (*dwarf1*). Each mutant affects tassel branch number (TBN) alone, and in double mutant combinations with *ramosa* mutant(s) we saw mostly additive effects on TBN and E(ear)BN. One exception was *Ts6*, which interacted synergistically to enhance the ra-63 mutant phenotype. We also directly tested the genetic interaction between *ra1* and its putative, direct target locus, liguleless1. Surprisingly, we saw no evidence for epistasis in this test, either. Our results reflect clear roles for JA (Jasmonic Acid) and GA (Gibberellins) in regulating inflorescence branching, by inhibiting and promoting branching respectively, and underscore other results from our lab supporting genetic complexity in which many pathways of small effect combine in mostly additive fashion to regulate inflorescence architecture.
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P200

Genetic interactions between maize *drooping leaf1*, *drooping leaf2* and leaf patterning mutants

(submitted by Josh Strable <jjs369@cornell.edu>)
Full Author List: Strable, Josh[1 2]; Unger-Wallace, Erica[1]; Vollbrecht, Erik[1]
[1] Department of Genetics, Development and Cell Biology, Iowa State University, Ames, IA USA 50011
[2] School of Integrated Plant Science, Cornell University, Ithaca, NY, USA 14853

Leaf architecture directly influences canopy structure, which affects yield. The basic plan of a mature leaf is established early in development, and this process is tightly regulated at the genetic level. A central problem in plant biology remains to understand the genes and their interactions that establish leaf architecture during organogenesis. We discovered and characterized a maize mutant with aberrant leaf architecture we named *drooping leaf1* (*drl1*). Pleiotropic mutations in *drl1* affect leaf length and width, leaf angle, and internode length and diameter. These *drl1* vegetative phenotypes are enhanced by the *drl2* enhancer locus in the Mo17 inbred, the *drl2-M* allele. We cloned the underlying genes and found that the paralogous loci encode maize CRABS CLAW co-orthologs, putative transcriptional regulators with zinc-finger and YABBY domains. The *drl* paralogs are co-expressed in incipient and emerging leaf primordia in B73 shoot apices. However, compared to expression of the *drl2-B73* allele in the shoot apex, *drl2-M* transcripts are highly reduced. A second *drl2* allele, produced by transposon mutagenesis, interacted synergistically with *drl1* mutants, corroborating functional redundancy between the paralogs. Here, we report on genetic interaction studies that investigate the relationship between the *drl* genes and genes that control leaf development. We find the *drl* genes network with other genes important for leaf patterning. Moreover, some genetic interactions altered leaf phyllotaxy, suggesting the *drl* genes regulate activity of the vegetative meristem in addition to their roles during leaf development. Taken together, these data demonstrate the necessity of the *drl* genes during leaf development and provide insight into their role as regulators of meristem activity and/or growth.

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P201

Genetic regulation of maize floral development

(submitted by Beth Thompson <thompsonb@ecu.edu>)
Full Author List: Nukunya, Kate[1]; Ding, Charlene[1]; de Luis Balaguer, Angels[2]; Sozzani, Ross[2]; Thompson, Beth[1]
[1] East Carolina University; Greenville, NC, 27858
[2] North Carolina State University, Raleigh, NC27695

Flowers have an essential role in plant reproduction and also produce fruits and seeds, which are a major food source. Grass flowers (florets) have a highly derived morphology and in addition to stamens and carpels contain the grass-specific organs locicules, palea and lemma. The genetic regulatory network that regulates floral development has been relatively well worked out in Arabidopsis and while some aspects of this regulatory network are conserved in maize and other grasses, much less is known about the regulation of floral development in the grasses. We are using multiple approaches to uncover the genetic regulatory network that controls floral development in maize, including double mutant analysis between known floral regulators and expression profiling of genes expressed in the upper and lower florets. We have also begun analysis and positional cloning of the classic semi-dominant mutant, *Polytypic1*, which has strong floral phenotypes in both the tassel and ear.

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P202

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

(submitted by Xiaoli Ma <xiaoli.ma@uni-tuebingen.de>)
Full Author List: Ma, Xiaoli[1]; Javelle, Marie[2]; Knauer, Steffen[2]; Schnable, Patrick[3]; Yu, Jianming[3]; Muehlbauer, Gary[4]; Scanlon, Mike[5]; Timmermans, Marja[1 2]
[1] Center for Plant Molecular Biology Biology, University of Tübingen, 72076 Tübingen, Germany.
[2] Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 11724, USA.
[3] Department of Agronomy, Iowa State University, Ames, Iowa 50010, USA.
[4] Department of Agronomy and Plant Genetics, University of Minnesota, St Paul, Minnesota 55108, USA.
[5] Division of Plant Biology, Cornell University, Ithaca, New York 14850, USA.

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. In addition, miRNA degredome sequencing data were produced, combined with information from the SAM atlas, we 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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P203

Genomic analysis of leaf cuticle development and functional diversity in maize

(submitted by Susanne Matschi <smatschi@ucsd.edu>)
Full Author List: Matschi, Susanne[1]; Vasquez, Miguel F[1]; Bourgault, Richard[2]; Qiao, Pengfei[3]; Scanlon, Michael J[3]; Molina, Isabel[2]; Smith, Laurie G[1]
[1] Section of Cell and Developmental Biology, University of California San Diego, La Jolla, CA 92093, USA
[2] Department of Biology, Algoma University, Sault Ste. Marie, ON P6A 2G4, Canada
[3] Plant Biology Section, School of Integrative Plant Science, Cornell University, Ithaca, NY 14853, USA

The cuticle is the outer physical barrier of plants and establishes an important interaction interface with the environment. This hydrophobic layer consists of the lipid polymer cutin embedded with and covered by waxes, providing protection against environmental stresses including desiccation, UV radiation, and pathogen attack. Thickness, structure, and chemical composition of the cuticle vary widely among plant species, and even within a species, depend on organ identity, developmental stage, and growth conditions. The functional contribution of the maize cuticle and its components to abiotic and biotic stress responses have been rarely studied so far. Moreover, the genetic regulation and impact of the cuticle on the adult plant, agronomically the most important growth phase, is largely unknown. We are characterizing the biogenesis of the adult maize leaf cuticle and its genetic basis, and aim to elucidate the impact of the mature cuticle on important agricultural traits. A first part of the project is the characterization of cuticle maturation along the adult leaf developmental gradient as measured by cuticle permeability and resistance to water loss. Changes in cuticle composition, analyzed by GC-MS, were mapped to the developmental gradient of the adult maize leaf. Ultrastructural differences of the cuticle among epidermal cell types are elucidated via transmission electron microscopy (TEM), and this analysis will be extended to different developmental stages along the mature leaf in the future. In collaboration with Michael Scanlon's lab (Cornell), we are conducting an epidermal-specific transcriptomic analysis, which will be related to the compositional changes along the leaf. In collaboration with Michael Gore's lab (Cornell), we are also in the process of conducting a genome wide association study (GWAS) to identify genetic loci and candidate genes controlling cuticular evaporation (CE) rate, assessed as the water loss over the leaf surface independent of stomatal transpiration.

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P204

Identification of a unique spectral signature of black layer formation in maize (*Zea mays* L.)

(submitted by Valerie Craig <craigv@uoguelph.ca>)
Full Author List: Craig, Valerie[1]; Lee, Liz[1]; Bowley, Stephen[1]; Earl, Hugh[1]; Berg, Aaron[2]
[1] University of Guelph, Department of Plant Agriculture; 50 Stone Road East; Guelph; Ontario; Canada; N1G 2W1
[2] University of Guelph, Department of Geography; 50 Stone Road East; Guelph; Ontario; Canada; N1G 2W1

Physiological maturity in maize is reached at the developmental stage black layer, where photosynthates are no longer able to move from photosynthetic tissues (source) into the developed grain (sink). Currently, there is no high-throughput method available for determining physiological maturity in maize for field-based phenotyping (FBP), although remotely sensed spectral data may offer a solution to this problem. The aim of this project is to determine if there is a unique reflectance signature associated with the loss of a carbon sink during maturation, which could act as a signal for black layer. We induced a source-sink imbalance in maize plants by prematurely terminating the primary carbon sink. Since black layer is the natural termination of the primary sink, this removal may mimic the physiological consequences of that process. Sink termination was induced in 4-row plots over a 5-week period by manually removing all developing ears within a plot. Plots were then measured with a dual-channel UniSpec which records narrow band reflectance from 350nm -1100nm. We identified that the four genotypes used in this study react differently to the sink removal treatments and that the spectral signatures of control plants change in unique sections of their curve as they reach physiological maturity. The goal of this work is to develop a spectral sensor which could attach to an unmanned aerial vehicle (UAV) to be used as a tool for high-throughput FBP to aid in breeding efforts to develop extremely short season maize hybrids.

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P205

Identification of cellulase inhibitors using corn-seed produced enzymes

(submitted by Hong Fang <hong.fang@smail.astate.edu>)
Full Author List: Fang, Hong[1]; Kandhola, Gurshagan[2]; Hood, Kendall R.[3]; Hood, Elizabeth E.[4]
[1] Molecular Biosciences, Arkansas State University, Jonesboro, AR 72401
[2] Biological & Agricultural Engineering, University of Arkansas, Fayetteville, AR 72701
[3] Infinite Enzymes, LLC, PO Box 2654, Arkansas State University, Jonesboro, AR 72401
[4] Arkansas State University Biosciences Institute and College of Agri. & Tech., Arkansas State University, Jonesboro, AR 72401

Lignocellulosic biomass has been regarded as a sustainable feedstock for advanced biofuel production. However, multiple difficulties remain in the conversion of lignocellulosic feedstock to sugars and subsequent fuels and thus impede the development of advanced biofuels. One of the most serious problems is low cellulase efficiency due to inhibition by breakdown products involved in feedstock conversion. Even though the maize seed expression system has been used to produce economical and efficient cellulases, the inhibition of these enzymes by both exogenous inhibitors (generated during the pretreatment of cellulosic feedstock) and endogenous inhibitors (derived from maize expression system) prevents the highly efficient conversion of lignocellulosic feedstock. In order to eliminate potential inhibition, inhibitory compounds must be identified. Our approach is to construct two enzymatic analysis systems based on either 4-methylumbelliferyl-β-D-cellobioside (MUC) or cellulose powder with three recombinant cellulases (endocellulase E1, exocellulase CBH1 and exocellulase CBH2 expressed by transgenic corn seeds) and one commercial glucosidase (Novozyme 188, Sigma). With these two enzymatic analysis systems, inhibitors derived from either hot-water-pretreated pine bark or the transgenic corn seeds will be identified.

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P206

Identification of interacting proteins with NARROW ODD DWARF, a protein required for normal developmental pattern in maize

(submitted by Jazmin Abraham <abrahammj@berkeley.edu>)
Full Author List: Abraham, Jazmin[1]; Rosa, Marisa[1]; Lewis, Michael[1]; Hake, Sarah[1]
[1] Plant Gene Expression Center, University of California, Berkeley. 800 Buchanan St., Albany, CA., USA. 94710,

Maize is a very important agronomic crop and it is used as a genetic model in plant biology. Identification of the genetic basis of mutant phenotypes is helpful in breeding unique varieties. In this project, we are using a mutant affected in a number of important traits for maize yield as a tool to understand molecular mechanisms that control plant architecture. The *narrow odd dwarf* (*nod*) is an EMS mutant, and shows severe pleiotropic defects in vegetative and reproductive organs. The affected gene has been described as part of the CELL NUMBER REGULATOR family in maize, however it has not been characterized. It is interesting that the nod phenotype is genetic background dependent. NOD is an *Arabidopsis thaliana* MCA1 ortholog, which complements a Ca^{2+} transport defective yeast mutant and is a plasma membrane-localized protein. The *mca1* mutant doesn't have a phenotype affected in development. To know the pathways within which NOD functions, we have done identification of NOD protein interactors by immunoprecipitation (IP) and Y2H assays and some interactions have been confirmed by BiFC and CoIP in *N. benthamiana*. In addition, we have carried out an RNA-seq analysis comparing wt and *nod* siblings in two different genetic backgrounds. Protein-protein interaction analysis showed proteins related to Ca^{2+} transport, hormone signaling and stress response. Analysis of differentially expressed genes from the RNA-seq showed overrepresentation of hormone metabolism, signaling, stress and cell wall related genes. These results are giving new insights about the NOD maize membrane protein and its interaction with important regulators involved in energy homeostasis, growth, stress response and development. All together, the project will help to elucidate the relationship between plant architecture and environmental response.

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P207

Identification of novel molecular components involved in the tillering regulation network of maize

(submitted by Yuguo Xiao <yuguo_xiao@byu.edu>)
Full Author List: Xiao, Yuguo[1]; Govindarajulu, Rajanikanth[2]; Bartlett, Madelaine[3]; Kelly, Jacob[1]; Kimball, Alex[1]; Longstaff, Muriel[1]; Hawkins, Jennifer[2]; Whipple, Clinton[1]
[1] Department of Biology, Brigham Young University, Provo, UT 84602,USA
[2] Department of Biology, West Virginia University, Morgantown, WV 26505,USA
[3] Department of Biology, University of Massachusetts Amherst, Amherst, MA 01003,USA

Shoot branching or tillering is an important agricultural trait that affects grain yield and biomass production. Commercial maize inbreds produce no tiller or few tillers. However, teosinte, the wild ancestor of maize, shows profound tillering phenotype. *teosinte branched1* (*tb1*) and *grassy tillers1* (*gt1*) are two of the major genes that have been selected during domestication of maize from teosinte to increase the apical dominance of maize main stalk and modulate maize tiller numbers. Mutants with loss-of-function of *tb1* or *gt1* exhibit increased tiller numbers. Both *tb1* and *gt1* act in a pathway to regulate tiller development in response to shade signals. To explore the additional genes involving in the *tb1-gt1* tillering network, we performed EMS-mutagenesis to screen potential suppressors and enhancers of *tb1* and *gt1*. In total, we identified 10 potential suppressors of *tb1* and 4 *of gt1*, and 2 *tb1* potential enhancers. Currently we are working on the cloning and characterization of these suppressors and enhancers. One of *tb1* suppressors was confirmed to be a new allele of *baf1* (*BARREN STALK FASTIGIATE1*); one of *tb1* enhancers was mapped to a 6Mb region of Chr1.

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P208

Identifying early events in proximal-distal patterning of the maize leaf

(submitted by Josh Strable <jjs369@cornell.edu>)
Full Author List: Strable, Josh[1]; Johnston, Robyn[1]; Leiboff, Samuel[2]; Federici, Silvia[1]; Hake, Sarah[2]; Sylvester, Anne[3]; Scanlon, Michael[1]
[1] School of Integrated Plant Science, Cornell University, Ithaca, NY, USA 14853
[2] Plant Gene Expression Center, USDA-ARS, Albany, CA, USA 94710
[3] Department of Molecular Biology, University of Wyoming, Laramie, WY, USA 82071

A central topic in plant biology is how leaves are patterned during organogenesis. Maize is a tractable system to address this subject: the distal blade, proximal sheath, and ligule and auricle boundary are delineated early in leaf development. Previously, we identified transcriptomic signatures of early proximal-distal patterning by comparing developing pre-blade, pre-sheath and pre-ligule at plastochron 7 (P7) using laser microdissection RNAseq (LM-RNAseq). Several candidate genes implicated in the regulation of ligule initiation were also expressed at the base of younger primordia (e.g., P4). Based on these observations, we hypothesized that positioning of the preligule boundary is set up by early-onset patterning events in emerging primordia, which, in turn, could be important for proper boundary placement of the preligule band in later-staged primordia. We explored this hypothesis through LM-RNAseq profiling four contiguous proximal-distal microdomains, beginning at the base of P4 primorida. We identified 1,045 differentially expressed genes that partition into 25 nodes using self-organizing map analysis. Approximately 20% of differentially expressed genes in the P4 primorida are shared with genes identified in the P7 pre-ligule, including those confirmed previously to be expressed in the developing ligule. Nearly one-sixth of these genes are putatively bound and modulated by KN1. Many genes shared between the P4-P7 pre-ligule datasets are enriched in the two most proximal contiguous segments from our P4 sampling, suggesting organ boundary positioning may occur along a gradient during the leaf development. We are comparing expression patterns of candidate genes in *liguleless* mutants and maize diversity inbred lines to broaden our understanding of when and how proximal-distal patterning is specified in leaf primordia.

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P209

Investigating lateral organ boundary formation using tassel branch mutants

(submitted by Martin Alexander <martinalexander@berkeley.edu>)
Full Author List: Alexander, Martin[1]; Lewis, Mike[1]; Richardson, Annis[1]; Hake, Sarah[1 2]
[1] Department of Plant and Microbial Biology, University of California, Berkeley, CA 94720, USA
[2] Plant Gene Expression Center, Agricultural Research Service, United States Department of Agriculture, Albany, CA 94710, USA

In maize, lateral organs are borne along a central axis. The size, number and angle of these organs dictate overall productivity. At the time of flowering, the shoot apical meristem (SAM) transitions to the male inflorescence meristem which forms the tassel, a spike with branches at the base and spikelets borne along these primary and secondary axes. Tassel architecture is a useful model to study branch development, in part due to their large size making traits easy to score and quantify. A transitional series of branching events splits the inflorescence meristem to form branch, spikelet pair, spikelet, and floral meristems, while the branch meristem grows out to recapitulate this transitional series. Indeed, all lateral organs originate from the SAM in a similar manner, with a boundary formed around a group of founder cells pre-determined to become the next lateral organ. We are using several classical tassel mutants to explore the genetic framework for the formation of the boundary that separates this lateral cluster of meristem cells from the central meristem, launching their new trajectory as a branch primordium. One of these mutants is the dominant *Wavy auricle in blade1 (Wab1-R)*, which has ectopic auricle tissue in the leaf blade, and an excessively wide tassel branch angle due to its regulation of *liguleless1*. The recessive alleles *wab1-rev (revertant)* and *wab1-bad (branch angle defective)* produce normal auricles but have an upright and reduced tassel branch phenotype. These mutants provide us with a unique tool for studying lateral organ boundary formation given the loss and gain-of-function alleles, antibodies and distinct phenotypes.

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

P210

Investigation of the redundancy of transcription factors expressed during seed development

(submitted by Fei Ge <gefei511@waksman.rutgers.edu>)
Full Author List: Dong, Jiaqiang[1]; Ge, Fei[1]; Messing, Joachim[1]
[1] Waksman Institute, Rutgers, The State University of New Jersey, Piscataway, NJ 08854-8020

Maize is an excellent cereal model for research on seed development due to its relatively large size for both embryo and endosperm. Recently, high-resolution spatial and temporal transcriptome studies of B73 maize seed development have been reported, with hundreds of transcriptional factors (TF) expressed. However, the actual role of these TFs is largely unknown. Whereas gene knockout mutants have been used to examine whether a gene is essential for proper development, such studies are frequently hampered by gene copy number variation and the redundancy of gene functions. In fact, different transposon element systems have been used to generate gene knockout collections in maize without a phenotype in its heterozygous state. Moreover, the junctions of several Mu or Ds transposon insertion libraries have been indexed with sequence information, which provide us with a critical resource to study seed development with a reverse genetic method. Here, we used phenotypical analysis of 40 transcription factors that are expressed during kernel development to uncover their roles. Because the maize genome was duplicated during its evolution, we used a bioinformatic approach to selected genes that were homologous to known genes and shared a similar expressional pattern with the selected maize TFs. The knockouts of the selected genes were combined by crossing to construct double mutants. We discovered that these 40 double mutants showed a normal kernel phenotype. Thus, despite induced expression in kernel development, our results indicate that these genes are not essential for kernel development or are compensated by other redundant genes. These sets of data form the basis for additional studies on the regulation of seed development.

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P211

Maize cell genomics: Functional cell/tissue-specific analysis using fluorescent protein lines and a two-component transactivation system

(submitted by Edgar Demesa-Arevalo <edemesaa@cshl.edu>)
Full Author List: Demesa-Arevalo, Edgar[1]; Luo, Anding[2]; Wu, Qingyu[1]; Je, Byoung Il[1]; Steinkraus, Holly[2]; Skopelitis, Tara[1]; Krishnakumar, Vivek[3]; Choi, Yongwook[3]; Chan, Agnes[3]; Sylvester, Anne W.[2]; Jackson, David[1]
[1] Cold Spring Harbor Laboratory, 1 Bungtown Road, Cold Spring Harbor, NY11724, USA
[2] Department of Molecular Biology, 1000 East University Ave, University of Wyoming, Laramie, WY 82071, USA
[3] The J. Craig Venter Institute, 9712 Medical Center Drive, Rockville, MD20850, USA

Functional genomics tools are currently needed to leverage the quantity of sequence data being generated in maize. To provide resources for functional study, we have generated over 100 stable, natively expressed, fluorescent protein (FP) fusion lines that mark most of the common subcellular compartments in maize. These lines are publicly available via our project website (http://maize.jcvi.org/cellgenomics) and have been used by the maize research community for developmental, physiological and functional studies. We also developed an LhG4 two-component transactivation system to drive cell, tissue and organ-specific expression. Selected promoters activate expression of the LhG4 transcription factor, which in turn will transactivate genes of interest driven by the pOp promoter in responder lines. Currently, 34 driver constructs have been produced to drive expression in shoot and inflorescence meristems, leaves, embryo or roots, using tissue-specific promoters. Eight responder constructs are currently completed, including FLOWERING LOCUS T like *Zea mays* CENTRORADIALIS 8 (ZCN8), *Zea mays* FON2-LIKE CLE PROTEIN1 (*Zm*FCP1), *Zea mays* CLV3/ESR-related7 (*Zm*CLE7), EMBRYO-SURROUNDING REGION 2C1 (ESR2C1, another *Zea mays* CLE peptide) and MALE STERILE CONVERTED ANTHER 1 (MSCA1), a maize glutaredoxin involved in the establishment of phyllotaxy. Transactivation has been successfully tested using this system: for example, the ZCN8 responder driven by the constitutive promoter driver from maize ELONGATION FACTOR 1 alpha phenocopies the early flowering phenotype of ZCN8 overexpression. This system has been used to test new hypotheses in meristem regulation by differentiated cells; expressing *Zm*FCP1 using a leaf primordia-specific driver line strongly inhibited meristem growth, confirming this feedback regulation.
Additionally, we are using our root tissue-specific marker lines p*Zm*WOX5:4XNLS-tagRFPt, p*Zm*SHR:4XNLS-tagRFPt and p*Zm*SCR:4XNLS-tagRFPt to standardize Fluorescence Assisted Cell Sorting (FACS) protocols, in collaboration with the Birnbaum Lab (NYU). Once optimized, other inflorescence meristem lines will be used for cell/ tissue-specific functional studies.

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P212

Maize embryo morphogenesis: a mutational and confocal analysis

(submitted by Dale Brunelle <dale.brunelle@und.edu>)
Full Author List: Brunelle, Dale C.[1]; Clark, Janice K.[1]; Sheridan, William F.[1]
[1] University of North Dakota; Biology Dept.; Grand Forks, North Dakota, 58202

The maize zygote normally develops over approximately 45-days passing through the proembryo, transition, coleoptilar, and stage1 (first leaf primordium) morphogenetic stages followed by the iterative formation of additional leaf primordia during stages 2-6 according to Abbe and Stein (1954). Using EMS treatment of maize pollen, we have produced more than 50 lethal embryo mutants that have no obvious effects on endosperm development except for some reduction in kernel size in some cases. Our dissections to date of mutant embryos in mature kernels show that about one-third of the mutations are blocked from the late proembryo through the late transition stage. During this period the embryo shifts its axis of symmetry, and the internal precursor region of the shoot apical meristem becomes evident in sectioned material. Most of the other mutants are blocked at abnormal coleoptilar stages. Germination tests of 25 mutant embryos for each of 44 mutants revealed that almost all mutants had zero germination or only a few germinated which were likely misidentified kernels. Our earlier work with putative Mutator-induced embryo mutations identified a larger proportion of mutations that were blocked in the coleoptilar and later stages of development (Clark and Sheridan 1991, Sheridan and Clark 1993). We are examining selected gene expression in normal and mutant embryos using confocal microscopy with 11 fusion gene constructs: PIN1, DR5, pWus, TCS, ab1, Rab17, MreIIB, PRK, HIS, ZmPeri, and BES1. Gene expression of these constructs in normal embryo development at 8, 9, 10, 12, and 15 days after pollination are currently being evaluated. Also, twelve *emb* mutants have been crossed by Pin1, DR5, pWus, and TCS. Samples were taken from these materials 30 days after pollination and the embryos examined using confocal microscopy.

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P213

Maize *Ufo1* mutant shows developmental defects that may be associated with stomata deformities

(submitted by Debamalya Chatterjee <debamalya1989@gmail.com>)
Full Author List: Chatterjee, Debamalya[1]; Wittmeyer, Kameron[1]; Cui, Jin[1]; Lee, Tzuu-fen[2 4]; Meyers, Blake[2 3]; Chopra, Surinder[1]
[1] Department of Plant Science, The Pennsylvania State University, University Park, PA 16802
[2] Department of Plant and Soil Sciences, University of Delaware, Newark, DE 19711
[3] Division of Plant Sciences, University of Missouri, Donald Danforth Plant Science Center, St. Louis, MO 63132
[4] Pioneer Hi-Bred Inc., Johnston, Iowa

The *Unstable factor of orange1* (*Ufo1*) is a dominant modifier of the expression of maize *pericarp color1* (*p1*) gene. The *Ufo1* plants show severe developmental defects in particular, phenotypes that resemble constitutive drought stress in absence of drought. We found that *Ufo1* plants had reduced transpiration and leaf conductance, indicating stomata closure or defective stomata. Microscopic studies of the epidermal cells revealed abnormal stomata, which may form because of defective cytokinesis during stomata formation. The stomata development involves polarization of subsidiary mother cell (SMC) towards guard mother cell (GMC) of stomata. The *Ufo1* plants appear to be unable to form normal subsidiary cells from SMC. In extreme cases frequency of deformed stomata is high enough to distort pavement cell layers. In maize, the *pan1* and *pan2* mutants are extensively studied for stomata defect. The *pan1* gene is known to promote pre-mitotic polarization of SMC and localization of leucine-rich repeat receptor like protein on the contact site of SMC and GMC. Our current work also explores the *Ufo1* RNAseq database to detect any differential expression of stomatal development related genes like *pan1*, *pan2*, *pin1*, *pin2* and other genes in mutant and the wild type. These analyses will therefore help unravel yet an unknown control of stomata development that may in turn allow us to solve the mystery of developmental defects in *Ufo1* mutant.

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P214

Maize *YABBY* genes *drooping leaf1* and *drooping leaf2* regulate floral development

(submitted by Josh Strable <jjs369@cornell.edu>)
Full Author List: Strable, Josh[1 2]; Vollbrecht, Erik[1]
[1] Department of Genetics, Development and Cell Biology, Iowa State University, Ames, IA USA 50011
[2] School of Integrated Plant Science, Cornell University, Ithaca, NY, USA 14853

Floral units in cereal crops produce grain, directly impacting yield. A key question in plant biology remains what factors regulate floral development. We recently cloned the maize *CRABS CLAW* co-orthologs, *drooping leaf1* (*drl1*) and *drl2*, members of the *YABBY* family of transcriptional regulators. Mutations in *drl1* result in a sterile ear in which pistillate florets display ectopic unfused carpels that fail to enclose a proliferative nucellus. In *drl1* mutant tassels, staminate florets have extra stamens and retain fertile anthers. These *drl1* floral phenotypes are enhanced by the *drl2* enhancer locus in the Mo17 inbred, the *drl2-M* allele. A second *drl2* allele, produced by transposon mutagenesis, interacted synergistically with *drl1* mutants, corroborating functional redundancy is at play between the paralogs. The *drl* paralogs are co-expressed in lateral primordia initiated by the floral meristem, but not within the floral meristem. This expression pattern coupled with indeterminate floral meristems observed for *drl* mutants indicate these genes likely regulate floral meristem activity and impose meristem determinacy through a non-cell autonomous mechanism. Additionally, *drl* expression patterns are conserved in the Panicoideae, suggesting shared function(s) in related grasses. Genetic interaction analyses of *drl* mutants with maize floral mutants indicate the *drl* genes are required throughout floral development, illustrating their importance for proper floral patterning in maize.

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P215

Manipulation of heterotrimeric G proteins alters maize development, immune responses and agronomic traits

(submitted by Qingyu Wu <qwu@cshl.edu>)
Full Author List: Wu, Qingyu[1]; Char, Si Nian[2]; Yang, Bing[2]; Jackson, Dave[1]
[1] Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724
[2] Department of Genetics, Development and Cell Biology, Iowa State University, Ames, Iowa 50011

The heterotrimeric G protein complex, containing Gα, Gβ and Gγ subunits, plays important roles in plants, however is not as well understood as in animals. We have shown that mutants of the maize Gα-subunit *COMPACT PLANT2 (CT2)* have enlarged shoot apical meristems (SAMs) and fasciated ears. In addition to CT2, the sole canonical Gα of maize, there are three eXtra Large G proteins (XLGs), which have a domain with homology to Gα as well as additional domains. Maize also contains one Gβ and six Gγ genes. To understand the functions of these G protein subunits, we generated both gain-of-function and loss-of-function mutants. We first generated a constitutively active version of CT2 (CA-CT2) by introducing the Q223L mutation in the context of a native construct. A yeast-3-hybrid study suggested that the Q223L mutation abolished the interaction between CT2 and the Gβγ dimer, confirming that $CT2^{Q223L}$ is indeed constitutively active. Expression of *CA-CT2* in a *ct2* mutant background resulted in interesting phenotypes, including higher spikelet density and kernel row number, larger ear inflorescence meristems and smaller leaf angles compared with normal sibs. We also used CRISPR-Cas9 to knockout the *Gβ*-subunit gene, *ZmGB1* and the three maize *XLGs*. Both *Zmgb1* single and *xlg1,2,3* triple mutants were lethal at the seedling stage. The *Zmgb1* mutants showed over-accumulation of H_2O_2, constitutive activation of MAP-kinases, and up-regulation of *PR1 (PATHOGENESIS-RELATED 1)* and *PR5*, two immune marker genes. These results suggest that *ZmGB1* mutation caused autoimmune symptoms. The mechanism underlying the lethality of *xlg123* triple mutants remains to be unraveled. Interestingly, knocking out *XLGs* in *ct2* mutant background enhanced its dwarf phenotype. Further studies will focus on understanding the roles of Gβ and XLGs in maize development and immune responses.

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P216

Morphological and cell division phenotypes of maize katanin mutants

(submitted by Nicholas Miles <nichomiles@gmail.com>)
Full Author List: Miles, Nicholas W.[1]; Lau, Kin[2]; Weil, Clifford F.[2]; Wright, Amanda J.[1]
[1] University of North Texas; Denton, TX, USA 76203
[2] Purdue University; West Lafayette IN, USA 47907

The microtubule severing protein, katanin, is a multimeric protein that includes p60 catalytic subunits and p80 regulatory subunits. There are two p60 paralogs in the maize genome. A variety of mutations that affect the p60 paralogs, including those that cause premature stop codons, non-synonymous amino acid substitutions, and alternative splice variants, have been identified. We present a new p60 mutation combination that is hypothesized to create maize plants completely lacking katanin p60 function. We compare the morphological and cellular impacts of this loss of function mutation combination to phenotypes caused by other gain of function and partial loss of function mutation combinations.

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P217

Morphometric diversity and comparative development of sorghum inflorescences

(submitted by Samuel Leiboff <sleiboff@berkeley.edu>)
Full Author List: Leiboff, Samuel[1]; Hake, Sarah C.[1]
[1] Plant Gene Expression Center; 800 Buchanan Street; Albany, CA, 94710

Sorghum bicolor (L. Moench) is a drought-resistant relative of maize that generates a highly-branched inflorescence of perfect flowers called a panicle. Although a great deal is known about the genetic pathways that regulate the formation of the maize tassel, less is known about the sorghum panicle. Using a high-throughput imaging platform I analyzed the panicles from a 200-inbred subset of the sorghum association mapping panel (SAP). Using publically-available SNPs, I used a mixed-model GWAS to identify panicle morphology candidate genes. Natural diversity in sorghum panicle morphology did not identify orthologs of known, classical master regulators of maize tassel development. Instead, panicle morphology candidates implicated unexpected hormone transporters and transcription factor families. Further comparison of ontological events in sorghum panicle and maize tassel development by scanning electron microscopy (SEM) suggest that there may be many developmental differences in panicle and tassel formation. Ongoing transcriptomic and reverse genetic studies continue to explore the similarities and differences of inflorescence development in these closely-related species.

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P218

Natural variation in the unfolded protein response in maize

(submitted by Zhaoxia Li <zhaoxial@iastate.edu>)
Full Author List: Li, Zhaoxia[1]; Srivastava, Renu[1]; Tang, Jie[2]; Howell, Stephen[1 2]
[1] Plant Sciences Institute, Iowa State University, Ames IA 50011 USA
[2] Department of Genetics, Development and Cell Biology, Iowa State University, Ames IA 50011 USA

Adverse environmental conditions can interfere with the sensitive process of protein folding in the endoplasmic reticulum (ER), leading to the accumulation of misfolded proteins in the ER. This creates a condition called ER stress and elicits the Unfolded Protein Response (UPR), which upregulates the expression of a constellation of stress response genes. The UPR signaling pathway in plants has two arms – one which involves membrane-associated bZIP transcription factors (bZIP17 and bZIP28) and another involving IRE1-mediated splicing of bZIP60 transcription factor messenger RNA (bZIP60 mRNA). We screened maize NAM founder line seedlings in the laboratory for variation in production and splicing of bZIP60 mRNA in response to heat stress (37°C for 1hr). In response to heat stress, we observed that tropical lines show greater upregulation of unspliced bZIP60 mRNA and more accumulation of the spliced bZIP60 mRNA. We further pursued variation in RILs derived from a cross of one of the tropical lines (CML52) with B73. Association analysis showed that 46 of the SNPs contributed significantly to the upregulation of unspliced bZIP60 and 48 SNPs contributed to the spliced bZIP60 mRNA form. Phenotypic variation explained by individual SNPs ranged from 4%-10% for the unspliced form and from 3%-17% for spliced bZIP60. Interestingly, there was a large-effect association haplotype block located on chromosome 9, in the upstream region of bZIP60, suggesting that polymorphisms upstream of bZIP60 contribute significantly in this tropical line to the upregulation of bZIP60 expression in response to heat stress.

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P219

Nitrogen limitation reduces ear growth by complicated physiological and molecular mechanisms

(submitted by Xuexian Li <steve@cau.edu.cn>)
Full Author List: Li, Xuexian[1]; Han, Jienan[1]; Yu, Jiaojiao[1]
[1] 2 W Yuanmingyuan Ave, Dept of Plant Nutrition, China Agricultural University

Nitrogen (N) is one of the most important nutrients for crop production and global food security. How N limitation affects ear growth remains largely unclear at physiological and molecular levels. Our results showed that N limitation significantly inhibits ear longitudinal growth, tip kernel abortion, and subsequent yield losses. These N-limitation outcomes are due in part to disrupted homeostasis of amino acids and down-regulation of activities of enzymes involved in N and carbon metabolism. Consistent with significantly lower auxin concentrations in the maize ear, proteomic analysis revealed alterations of auxin metabolism and attenuation of auxin signaling. In particular, expression of AUX/IAA19 is dramatically upregulated in the maize ear under N limitation. GFP-tagged AUX/IAA19 is localized in the nucleus, and AUX/IAA19 is a negative transcriptional regulator. It potentially interacts with ARF4, ARF6, and RUM1. Overexpression of AUX/IAA19 significantly reduces the kernel number. Thus, AUX/IAA19, regulated by N status, is an important player in controlling ear and kernel development in maize.

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P220

nop1 and *nop2* are paralogous genes with likely functions in the maize male gametophyte

(submitted by Matthew Warman <warmanma@oregonstate.edu>)
Full Author List: Warman, Matthew D[1]; Colebrook, Sean[1]; Fowler, John[1]
[1] Oregon State University, Department of Botany & Plant Pathology, Corvallis, Oregon, 97331

Fundamental aspects of maize reproduction, including pollen tube tip growth, are poorly understood. The characterization of highly expressed genes in mature pollen allows for potential insight into these mechanisms. GRMZM2G372877 was previously identified in this group (Chettoor et al. 2014) and is now designated *nop1*, based on orthology with the rice *no pollen* (*Osnop*) gene (Jiang et al. 2005). A *Ds* insertion in *nop1* was found to have a male-specific reduced transmission rate (Colebrook et al. 2015). Here, additional data are presented supporting the hypothesis that both *nop1* and its paralog *nop2* function in the male gametophyte.
Combining the *nop1::Ds* allele with chromosome inversion 9b generated recombination-resistant plants with the *nop1::Ds* mutation linked to the *Wx1+* phenotype. Outcrossing to a *wx1* tester with varying amounts of pollen resulted in significant differences in transmission of *nop1::Ds*, providing further evidence that loss of *nop1* function affects pollen competiveness. Stable, derivative *nop1* alleles from crosses with *Ac* active lines were identified using PCR to detect *Ds* footprints. To test for phenotypic reversion, derivatives with no frameshift will be identified using a similar method.
In rice, homozygotes for a large multi-gene deletion containing the *Osnop* gene were associated with shriveled anthers and late stage pollen degeneration. In contrast, no visible anther phenotypes in homozygous mutant *nop1* plants were observed. This could be the result of redundant function between *nop1* and GRMZM2G470666, a paralogous gene tentatively named *nop2*. Similar to its paralog, *nop2* showed increased expression in mature pollen relative to other tissues. Characterization of a validated *Mu* insertion from the UniformMu population is ongoing. Structural analysis of NOP protein family sequences showed potential sites for binding both calcium and phosphoinositide. This suggests a hypothesis linking *nop1* to tip growth, a function that would require interaction with these central signaling molecules.

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P221

Opaque1: A myosin XI mutant influencing polarization in maize during asymmetric cell division

(submitted by Janette Mendoza <jmendo01@unm.edu>)
Full Author List: Mendoza, Janette[1]; Facette, Michelle[1]
[1] UNM Biology, Castetter Hall 1480 MSC03-2020, 219 Yale Blvd NE, 1 University of New Mexico Albuquerque, NM 87131-0001

Asymmetric cell division (ACD) is important because it determines cell fate and tissue patterning. However, many aspects of ACD in plants are still unclear. Stomatal development in maize has proven to be a useful model for understanding the ACD mechanism. Previous studies have identified several actors in subsidiary mother cell (SMC) polarization including BRK proteins (regulators of actin nucleation), PAN proteins (receptor- like molecules), and ROP (a GTPase). A dense actin patch also polarizes in SMCs, and the nucleus polarizes via an actin-based mechanism. After polarization the preprophase band marks the division plane, followed by cytokinesis. Mutations have also been identified in division plane establishment and maintenance (*dcd* and *tan*). *Opaque1* was previously identified as a maize myosin XI important for protein body localization in seeds[1]. Plants have two types of myosins: myosin VIII and myosin XI. Myosin XIs are required for organelle movement and cytoplasmic streaming. *Thus, we hypothesize that myosin is required for ACD in maize and has several potential roles in ACD*. We speculate myosins can play a role during the perception of the polarizing cue, during polarization of organelles in the cell, or during formation of the spindle alignment. Preliminary data showed *opaque1* has abnormal subsidiary cells. The shapes of the abnormal subsidiary cells closely resemble *dcd mutants*, rather than *pan* or *brk* mutants, suggesting *opaque1 may have defects post-polarization*. We are currently examining nuclear migration, actin, and PAN protein polarization in *Opaque1* mutants to determine if they have defects during polarization.

P222

Persistent homology: A mathematical framework to interpret complex growing plant topologies

(submitted by Mao Li <mli@danforthcenter.org>)
Full Author List: Li, Mao[1]; Jiang, Ni[1]; Duncan, Keith[1]; Chitwood, Daniel H.[1]; Topp, Christopher N.[1]
[1] Donald Danforth Plant Science Center; 975 N Warson Rd St. Louis, MO 63132 USA

The entire genome encodes the growing morphologies of shoots and roots. Yet, conventional phenotypic measures consider only isolated parts of the plant and thus quantify only a small amount of overall morphological variation, limiting our understanding of genetic and environmental conditioning of plant phenotypes. Persistent homology is a mathematical approach that can quantify variation in both topology and shape, across scales and time, and can comprehensively model plant morphology and understand the overall morphological changes induced by genes, evolution, and the environment. Here we describe persistent homology and present our applications to comprehensively quantify complex 3D growth (4D) patterns of maize root systems, in quantitative genetic analysis of 3D maize root architectures, and to identify a common genetic basis for leaf shape and root architecture in tomato. Finally, we describe our progress in combining X-ray Computed Tomography with Persistent Homology to capture and describe the plant form as it truly is: a single topology growing through time.

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P223

Prolonged growth duration partially compensates the growth rate reduction caused by mild drought and the timing of re-watering determines the cellular mechanism to resume growth

(submitted by Hilde Nelissen <hilde.nelissen@psb.vib-ugent.be>)
Full Author List: Takasaki, Hironori[1 2]; Wuyts, Nathalie[1 2]; Demuynck, Kirin[1 2]; De Block, Jolien[1 2]; Inze, Dirk[1 2]; Nelissen, hilde[1 2]
[1] VIB Center for Plant Systems Biology, Technologiepark 927, 9052 Gent
[2] Department of Plant Biotechnology and Bioinformatics, Ghent University, 9052 Gent

Drought occurs in almost all climatic regions and is responsible for tremendous yield losses. One of the first reactions of the plant sensing drought is a growth reduction. By studying the effects of drought on leaf growth of a B73 x H99 recombinant inbred population, we showed that the growth reduction is, at least partially, compensated by a prolonged duration of growth. This negative correlation between growth rate and duration was not observed under well-watered conditions as both processes were positively correlated with final leaf size in favorable conditions. To examine if the longer duration of growth imposed by drought stress can serve to keep the leaf in stand-by to resume growth when water becomes available again, we re-watered the plants at different time points during the growth. During early leaf growth, re-watering can fully restore the growth rate, allowing the leaf to grow similar to what was seen for well-watered plants. Re-watering plants during later stages of growth failed to fully restore the growth rate and even prolonged the duration of growth, which progressively became more pronounced as re-watering occurred during later time points. The underlying cellular mechanisms and the transcriptional adjustments, determined by RNAseq, at the different time points of re-watering will be discussed.

P224

Proteome communication between endosperm and embryo in maize

(submitted by Xixi Zheng <xxzheng@sibs.ac.cn>)
Full Author List: Zheng, Xixi[1]; Li, Qi[1]; Wu, Yongrui[1]
[1] National Key Laboratory of Plant Molecular Genetics, CAS Center for Excellence in Molecular Plant Sciences, Institute of Plant Physiology & Ecology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai 200032, China.

The double fertilization in flowering plants gives rise to the embryo and endosperm. Although they have different fates and biological functions, many evidences have shown that they appear to be developmentally coordinated through mutual communications. Here, we show that the embryo can sense and respond to the proteome alteration in the endosperm. Maize endosperm is the principal organ for the storage protein accumulation, and the main storage proteins in the endosperm are prolamins called zeins. Although the maize seed possesses a standardized proteome, mutations (like *o2, fl2*) or the *α-zein RNAi* can induce the proteome rebalancing, by which the loss of zeins is compensated by the increase of non-zein proteins, leading to a relatively constant total protein level in the seed. We characterized two major increased protein bands from the non-zein proteins in *o2* and *α-zein RNAi* seeds. Mass-Spec revealed that they are Globulin1 and Globulin2 (GLB1 and GLB2). RNA in situ hybridization showed that they are specifically expressed in the embryo. We compared the protein accumulation patterns in the embryo between the *α-zein RNAi* and WT and found that GLBs are the two most predominant increased proteins. In the triple mutant of *α-zein RNAi; glb1; glb2*, no new obviously increased proteins were observed in the embryo, indicating that GLBs are the specific proteins in the embryo responding to the proteome alteration in the endosperm. Transcript levels of *Glbs* are also dramatically enhanced, indicating that the proteome rebalancing is regulated in part at the transcriptional level. We proved that VP1 is able to strongly transactivate *Glbs* in the presence of ABA, but it is not induced in the proteome rebalancing, suggesting unknown factors may be responsible for the response. Currently, we are actively identifying the factors.

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P225

Reverse genetic approaches to understanding the role of auxin in maize development

(submitted by Joseph Struttmann <jwsgk9@mail.missouri.edu>)
Full Author List: Struttmann, Joseph[1]; Marshall, Kiley[1]; Coats, Diana[1]; Withee, Jacob[1]; Malcomber, Simon[3]; Gallavotti, Andrea[2]; McSteen, Paula[1]
[1] Division of Biological Sciences, University of Missouri. Columbia, MO 65211
[2] The Waksman Institute of Microbiology, Rutgers University. Piscataway, NJ 08854
[3] Department of Biological Sciences, California State University. Long Beach, CA 90840

The growth hormone auxin regulates nearly all aspects of plant development. Therefore, a better understanding of the genes controlling auxin biosynthesis, transport, and perception is fundamentally important to basic plant biology with applications in crop improvement. Previous research has demonstrated both conservation and diversification of the role of auxin in maize and Arabidopsis development. We are using maize vegetative and reproductive development as a model to further understand how auxin regulates development using both forward and reverse genetic approaches.
Phylogenetic analysis of 15 gene families controlling auxin biosynthesis, transport and response illustrates complex relationships amongst monocot and eudicot clades. Reverse genetic analysis has confirmed 103 transposon insertions (from the UniformMu and Mu-Illumina collections) in 47 genes. Phylogenetic and expression analyses coupled with higher order mutant phenotyping are revealing a more complex network than previously expected. Results from the vanishing tassel2 (vt2), ZmPIN, and ZmTIR/AFB gene families involved in auxin biosynthesis, transport, and perception respectively, will be presented.

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P226

Screening for potential binding partners of the DNA repair protein Rad51

(submitted by Claire Milsted <milst023@umn.edu>)
Full Author List: Milsted, Claire[1]; Dai, Bo[1]; He, Yan[2]; Dukowic-Schulze, Stefanie[1]; Sundararajan, Anitha[3]; Pillardy, Jaroslaw[4]; Kianian, Shahryar F[5]; Mudge, Joann[3]; Pawlowski, Wojciech P[2]; Chen, Changbin[1]
[1] Department of Horticultural Science, University if Minnesota, St. Paul, MN, 55108, USA
[2] Department of Plant Breeding and Genetics, Cornell University, Ithaca, NY 14853, USA
[3] National Center for Genome Resources, Santa Fe, NM, 87501, USA
[4] Computational Biology Service Unit, Cornell University, Ithaca, NY 14853, USA
[5] Cereal Disease Laboratory, USDA-ARS, St. Paul, MN 55108, USA

Rad51 is a conserved eukaryotic protein, involved in DNA repair in both somatic and meiotic cells, which plays a critical role in plant homologous recombination and immune response. Several other DNA repair molecules, such as DMC1 and BRCA2, are known to interact with Rad51; there are likely other molecules partnering with Rad51 which remain unknown. Our current aim is to identify the proteins with which Rad51 may interact in vivo and to further understand Rad51's function in maize. We present in this poster the results of a phage peptide display using a 16-mer peptide library designed to identify potential binding partners of the Rad51 protein in vitro. 24 phage colonies showed positive reaction against the denatured Rad51 protein, which led to the identification of 11 sequences with potential interaction with Rad51. Blast results showed several maize proteins with peptide sequences similar to the selected peptides. These can be sorted briefly into three categories according to annotation: 1) DNA- or RNA- binding, including DNA repair-associated proteins; 2) transcription regulator, disease and pathogen response proteins; and 3) unknown proteins. Further tests of those candidate proteins using reverse genetic approaches will help us to understand the mechanisms of Rad51's function inreproductive development and pathogen response.

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P227

Tasselseed5, a classic maize mutant, encodes a wound-inducible CYP94B3

(submitted by China Lunde <lundec@berkeley.edu>)
Full Author List: Lunde, China[1]; Leiboff, Samuel[1]; Hake, Sarah[1]
[1] UC Berkeley Plant Gene Expression Center, Albany, CA 94710

Tasselseed5 (Ts5) is a classic maize mutant and appears on a linkage map made by Emerson in 1932. Using current genetic maps of Chr4, we fine-mapped *Ts5* and found repressed recombination at the locus. Using a transcriptomics approach, we found ectopic 5 prime RNA reads of a CYP94B gene ectopically expressed in 9-12mm *Ts5* tassels and within our mapping interval. CYP94B3 enzymes catalyze the oxidative catabolism of JA-Ile during recovery from JA response. *Ts5* mutants fail to abort carpels in tassels as well as lower florets of the ear. We observed that the *Ts5* phenotype is completely feminized in Mo17 but this is suppressed in B73. To explore this natural variation, we mapped 10 QTL affecting feminization of *Ts5*. One QTL overlies *tasselseed2*, a gene known to participate in sex determination (DeLong et al. 1993 Cell 74:757-768), and having a greater than 4 fold reduction in *Ts5* tassels. An Arabidopsis ortholog was found to be wound-inducible (Koo et al. 2011 PNAS 108:9298-9303); we tested this possibility and found that both *Ts5* and *ts2* are wound-inducible and misexpressed in wounded *Ts5* leaves. In wildtype maize, *Ts5* is highly expressed in anthers, suggesting that high levels of JA-Ile inhibit stamen development.

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P228

The DNA binding landscape of maize auxin response factors

(submitted by Andrea Gallavotti <agallavotti@waksman.rutgers.edu>)
Full Author List: Gallavotti, Andrea[1 2]; Liu, Qiujie[1]; Sidharth, Sen[3]; Trupti, Joshi[3 4]; Lu, Zefu[5]; Schmitz, Robert[5]; Galli, Mary[1]
[1] Waksman Institute of Microbiology, Rutgers University, Piscataway, NJ, USA
[2] Dept. of Plant Biology, Rutgers University, New Brunswick, NJ, USA
[3] Informatics Institute, University of Missouri Columbia, Columbia, MO, USA
[4] Dept. of Molecular Microbiology and Immunology, School of Medicine, University of Missouri Columbia, Columbia, MO, USA
[5] Dept. of Genetics, University of Georgia, Athens, GA, USA

Auxin is an essential hormone for plant developmental pathways. We recently showed that auxin signaling modules containing the Aux/IAA proteins BARREN INFLORESCENCE1 (BIF1) and BARREN INFLORESCENCE4 (BIF4) are essential for the early steps of organogenesis in maize inflorescences (Galli et al., PNAS 2015). In order to investigate the downstream targets of these protein complexes, we employed a recently developed in vitro transcription factor DNA binding technique called DAP-seq (O'Malley et al., Cell 2016). Using DAP-seq, we analyzed the genome-wide DNA binding profiles of different maize AUXIN RESPONSE FACTORs (ARFs) that were found to be co-expressed with BIF1 and BIF4 in the early stages of inflorescence development. We identified thousands of ARF binding sites in the maize genome and discovered significant differences in binding site preference and peak distribution among ARFs belonging to different phylogenetically conserved clades. The genome-wide binding profiles of ARFs further allowed us to investigate the binding site architecture of Auxin Responsive Elements (AuxREs) in a genomic context as well as to identify novel downstream target genes. We will show that DAP-seq represents a powerful, low cost alternative for genome-wide high-throughput analysis of transcription factor DNA binding in maize.

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P229

The *dosage-effect defective kernel1* (*ded1*) transcription factor locus intersects genome dosage and imprinting regulation of endosperm development

(submitted by Janaki Mudunkothge <jmudunkothge@ufl.edu>)
Full Author List: Mudunkothge, Janaki S.[1]; Char, Si Nian[2]; Zhang, Junya[1]; Spielbauer, Gertraud[1]; Baier, John[1]; Yang, Bing[2]; Settles, A. Mark[1]
[1] Horticultural Sciences Department and Plant Molecular and Cellular Biology Program, University of Florida, Gainesville, FL 32611
[2] Department of Genetics, Development and Cell Biology, Iowa State University Ames, IA 50011

Seed size reflects the amount of nutrient reserves allocated to individual progeny. Mature kernel size is set, in part, during endosperm cell growth and differentiation. The maternal and paternal genomes of the endosperm have unequal contributions to endosperm development due to differences in ploidy as well as imprinted, epigenetic regulation. Hundreds of imprinted genes have been discovered, but functional roles are defined for a handful of these genes. We screened defective kernel mutations from the UniformMu population for seed weight dosage-effects and identified the *ded1* imprinted locus. Heterozygotes resulting from *ded1* gametes inherited through the male significantly reduce seed weight. Homozygous *ded1* mutants arrest embryo development and delay endosperm cell differentiation. Endosperm tissue culture suggests *ded1* prolongs a proliferative state. Map-based cloning of *ded1* identified an R2R3 MYB domain transcription factor gene with copia retrotransposon insertion. We generated additional *ded1* alleles through CRISPR/Cas9 targeted mutagenesis to confirm cloning. Published RNA-seq data indicate that *Ded1* is a seed specific gene with paternal biased expression in some inbred lines. We tested paternal biased expression of the W22 allele with an RT-PCR CAPS assay that suggests the paternal allele contributes 65-75% of the endosperm transcript level. These data are consistent with a dosage-effect in which one-third of the transcript is not sufficient to promote full grain-fill. RNA-seq comparing *ded1* and normal sibling endosperm found 7,154 of 25,333 detected genes were differentially expressed with 96% of them were down-regulated. These data suggest that DED1 normally functions to activate endosperm gene expression, promote endosperm differentiation, and promote nutrient allocation to the developing kernel. Paternal bias of *Ded1* expression is consistent with the parental conflict hypothesis, which explains imprinted gene expression as a competition for resources between parents. In this case, paternal expression of *Ded1* increases maternal support for the kernel.

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P230

The evolution of the *CLAVATA1/THICK TASSEL DWARF1*-like genes

(submitted by Jarrett Man <jaman@umass.edu>)
Full Author List: Man, Jarrett[1]; Liu, Lei[2]; Zadrozny, Tara[2]; Jackson, David[2]; Bartlett, Madelaine[1]
[1] University of Massachusetts Amherst, Bartlett Lab, 374 Morrill IV South, Amherst, MA 01003
[2] Cold Spring Harbor Laboratory, Jackson Lab, 1 Bungtown Rd Cold Spring Harbor, NY 11724

Meristem growth patterns contribute significantly to plant form. Indeed, meristem homeostasis genes have likely been domestication targets, as mutations in these genes can cause enlarged fruit size and increased yield. *CLAVATA1* (*CLV1*) in *Arabidopsis thaliana*, and one of its homologs in maize, *THICK TASSEL DWARF1* (*TD1*), are deeply conserved receptor-like kinase genes that regulate meristem homeostasis. *A. thaliana* has several close *CLV1* paralogs, some of which function to receive and transmit similar signals, but have different expression patterns and mutant phenotypes. *TD1* has long been known to be a *CLV1* homolog, and it also has many similar paralogs, though their function and exact evolutionary relationships to *TD1* remain unclear. Why are *TD1* duplicates conserved, and to what degree are their roles conserved across angiosperms? To begin addressing these questions, we constructed a phylogenetic hypothesis of all relationships in the *TD1* clade across the seed plants. In doing so, we discovered two previously undocumented maize *TD1* paralogs, confirmed the likely direct orthology of *TD1* and *CLV1*, and identified both ancient and recent gene duplications in the *TD1* clade. To investigate the evolution of gene regulation within the *TD1* gene clade, we are searching for both deeply conserved and grass-specific conserved non-coding sequences (CNSs). To determine how gene function has evolved following duplication, we are using CRISPR/Cas9 genome editing to knock out all known *TD1*-clade genes in maize. We hope to clarify the genetic architecture underlying the regulatory control and redundancy of these genes that affect meristem homeostasis.

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P231

The function of *Barren inflorescence3* in meristem initiation and maintenance in maize inflorescences

(submitted by Zongliang Chen <zlchen@waksman.rutgers.edu>)
Full Author List: Chen, Zongliang[1]; Li, Wei[1]; Menello, Caitlin[1]; Galli, Mary[1]; Gallavotti, Andrea[1]
[1] Waksman Institute of Microbiology, Rutgers University, Piscataway, NJ, USA, 08854-8020

The initiation and maintenance of meristems are essential for ear and tassel development, and can directly impact grain production. Meristem maintenance is established by a feedback loop between the *CLAVATA* and *WUSCHEL* genes to control stem cell homeostasis. We characterized a semi-dominant maize mutant, *Barren inflorescence3* (*Bif3*), that shows severe developmental abnormalities in ears and tassels, producing inflorescences devoid of spikelets due to a collapse of the inflorescence meristem as well as failure to initiate new axillary meristems. These severe inflorescence phenotypes suggest that *BIF3* is required for both the initiation and maintenance of meristem activity. Positional cloning was used to narrow the *Bif3* locus to a small region containing five predicted genes, one being *ZmWUS1*, a co-ortholog of the Arabidopsis WUSCHEL gene. *ZmWUS1* is strongly upregulated in *Bif3* mutants, and RNA *in situ* hybridizations show expanded and elevated expression of *ZmWUS1* in the organizing center of inflorescence meristems in *Bif3* mutants. Genetic analysis with other mutants affected in meristem maintenance such as *fea3* and *ct2* show more severe inflorescence meristem defects. We performed RNA-seq analysis on mutant inflorescences and identified hundreds of differentially expressed genes. Among these, enriched classes include protein kinase and homeobox genes that may work directly or indirectly with ZmWUS1 in initiating and maintaining maize reproductive meristems. Approaches to knock-out *ZmWUS1* in *Bif3* mutant plants using CRISPR/Cas9 and EMS are being carried out to confirm that *BIF3* encodes ZmWUS1.

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P232

The *HYDROPATTERNING1* (*HDP1*) locus affects root architecture and drought response in maize

(submitted by Wei Feng <wfeng@carnegiescience.edu>)
Full Author List: Feng, Wei[1]; Robbins, Neil[1]; Trontin, Charlotte[1]; Spencer, Dirk[1]; Rocheford, Torbert[2]; Dinneny, José[1]
[1] Carnegie Institution for Science; 260 Panama St.; Stanford, CA, USA 94305
[2] Purdue University; West Lafayette, IN, USA 47907

Water is a major factor that shapes root architecture in soil. When water availability is heterogeneous in soil, lateral root development is biased towards regions directly in contact with available water, and suppressed in regions where water is less available, a phenomenon termed hydropatterning. However, how plants sense the difference in water availability is still unknown. To further understand plant water relations, we uncovered phenotypic variation in hydropatterning within the *Zea mays* (maize) nested association mapping (NAM) population and sought to determine the associated genetic loci through quantitative trait locus (QTL) mapping. Phenotypic and genotypic characterization of recombinant inbred lines (RILs) allowed us to define a genetic interval of 1-Mb region associated with variation in hydropatterning, which we termed the *HYDROPATTERNING1* (*HDP1*) locus. Near-isogenic lines (NILs) with *HDP1* from different genetic backgrounds showed different root architectures under drought conditions during a field study, indicating a potential physiological role of lateral root hydropatterning in water-stress resistance. Further mapping of this QTL will likely uncover novel resources for understanding the molecular mechanisms for water perception.

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P233

The identification and characterization of genetic and physical interactors of RAMOSA3

(submitted by Xiaosa Xu <xxu@cshl.edu>)
Full Author List: Xu, Xiaosa[1]; Claeys, Hannes[1]; Vi, Son L[1 4]; Eveland, Andrea[2]; Skopelitis, Tara[1]; Nagasawa, Namiko S[1 5]; Goldshmidt, Alexander[1 6]; Sakai, Hajime[3]; Jackson, David[1]
[1] Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724, USA
[2] Donald Danforth Plant Science Center, St Louis, MO 63132, USA
[3] DuPont Pioneer, Agricultural Biotechnology, Wilmington, DE, 19803
[4] Vien Di Truyen Nong Nghiep, Hanoi, Vietnam
[5] Akita Prefectural University, Akita, Japan
[6] Monsanto, Woodland, CA 95695, USA

A fundamental understanding of plant meristem development requires insight of the molecular mechanisms regulated by different signaling pathways. *RAMOSA3* (*RA3*), which encodes a trehalose phosphate phosphatase (TPP) controls maize inflorescence shoot branching. Normally maize ears lack long branches, but *ramosa* mutant ears are branched. However, there is no direct connection found between the role of *RA3* in ear branching and its biochemical function as a TPP enzyme. A yeast-2-hybrid (Y2H) screen using RA3 to probe a library from maize inflorescences suggested that RA3 might mediate metabolic control of maize inflorescence meristem development by interacting with RNA binding proteins and transcription factors. To further probe the mechanism of RA3 function, we are conducting both ethyl methyl sulfonate (EMS) screening and immunoprecipitation-mass spectrometry (IP-MS) to identify the genetic and physical interactors of RA3, respectively. We have identified ~ 20 enhancer loci with > 20 ear branches (*ra3* mutant ears make an average of ~ 5 branches in B73). Among the enhancers, we found new alleles of *ramosa1* (*ra1*) and *ramosa2* (*ra2*) that were easily recognized by tassel architecture. Additionally, by mapping and sequencing, we identified one novel enhancer as *terminal ear1* (*te1*), which encodes an RNA binding protein involved in maize vegetative and reproductive development, and four enhancers as independent alleles of *trehalose phosphate phosphatase 4* (*tpp4*). These results validate the effectiveness of the EMS screen to find genetic enhancers of *ra3*. To identify RA3 associated proteins *in planta*, we are carrying out IP-MS for RA3 from developing maize inflorescences by using functional epitope tagged Flag-HA-RA3 maize lines. To overcome the limited tissue availability, we crossed Flag-HA-RA3 into a double mutant, branched *silkless*;*Tunicate* (*bd*;*Tu*) that transforms the maize ear into a “cauliflower” with over-proliferating meristems similar to ~ 2mm ear primordia. Candidate interacting proteins identified by IP-MS will be confirmed by independent methods. Their mutants from reverse genetics systems available in *Mutator* transposon systems, or made by CRISPR-Cas9 will be characterized.

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P234

The molecular identity of a novel enhancer of *Teosinte branched1*

(submitted by Jacob Kelly <j.a.kelly@byu.edu>)
Full Author List: Kelly, Jacob A[1]; Xiao, Yuguo[1]; Pickett, Brandon D[1]; Whipple, Clinton J[1]
[1] Department of Biology, Brigham Young University, Provo, Utah 84602

Teosinte branched1 (*tb1*) is a key regulator of lateral branch growth including tillers in maize and was a target of domestication. Tillers are vegetative branches that increase biomass. Recently, we discovered a novel maize mutant from a genetic screen for *tb1* modifiers which we call *enhancer of teosinte branched1* (*entb1*). When combined with *tb1*, *entb1* causes a dramatic increase in the number of tillers present in maize. Regardless of the presence of *tb1*, *entb1* also causes leaves with a decreased width, and shortened plant height. To determine the molecular identity of *entb1*, we created a large F2 mapping population from which we identified 97 homozygous *entb1* mutants. We extracted DNA from leaf samples of these plants, and after pooling the DNA samples, sequenced by paired-end 125bp Illumina HiSeq technology. Following a bioinformatics pipeline similar to Mutagenesis to Uncover Targets by deep Sequencing (MUTseq), we are in the process of mapping and determining the molecular identity of *entb1*. Little is known about the *tb1* genetic network, and we expect the cloning of *entb1* and subsequent research to characterize its function to provide new insights into a key pathway of tiller regulation.

Funding acknowledgement: National Science Foundation (NSF)

P235

The *rapunzel* (*rzl*) genes regulate growth suppression in maize florets

(submitted by Harry Klein <hrklein@umass.edu>)
Full Author List: Klein, Harry R[1]; Whipple, Clinton J[2]; Bartlett, Madelaine E[1]
[1] Department of Biology, University of Massachusetts, Amherst, MA 01002
[2] Department of Biology, Brigham Young University, Provo, UT 84602

Growth suppression is a fundamental process in the development of plant form, including in the tassel and ear of maize. Both tassel and ear florets are initially hermaphroditic, but programmed cell death of carpels in the tassel, and growth suppression of stamens in the ear leads to the development of morphologically distinct male and female florets. One major regulator of growth suppression, *grassy tillers1* (*gt1*), suppresses axillary branching and partially suppresses carpel growth in the tassel. While *gt1* acts to partially suppress carpel growth in the tassel, other genes acting with *gt1* to suppress carpel growth are unknown. To identify other genes that act with *gt1* to regulate carpel growth suppression and organ abortion, we conducted a *gt1* EMS enhancer screen. We identified 3 novel *gt1* enhancers, which we named the *rapunzel* (*rzl*) mutants. *rzl* mutants have increased carpel growth in the tassel compared to *gt1* mutants. In addition, lower ear florets are not suppressed in some *rzl* mutants. We developed a next-generation sequencing-based mapping strategy (Pool-Seq) to clone the *rzl* genes, and have localized two of the *rzl* genes to chromosomal regions. Future characterization of the *rzl* genes will elucidate novel players in the regulation of growth suppression and programmed cell death in maize floral development.

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P236

The *required to maintain repression12* locus provides a novel mechanistic link between paramutation and developmental gene regulation in *Zea mays*

(submitted by Natalie Deans <deans.11@osu.edu>)
Full Author List: Deans, Natalie C.[1]; Giacopelli, Brian[1]; Hlavati, Daniel C.[1]; McCormic, Emily J.[1]; Addo-Quaye, Charles A.[2]; Dilkes, Brian P.[2]; Hollick, Jay B.[1 3]
[1] Department of Molecular Genetics, The Ohio State University, Columbus, OH
[2] Department of Biochemistry, Purdue University, West Lafayette, IN
[3] Center for RNA Biology, The Ohio State University, Columbus, OH

In *Zea mays* (maize), paramutations facilitate meiotically heritable changes in gene regulation for certain alleles of *purple plant1*, a gene encoding a transcription factor required for anthocyanin production[1]. 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 passed on in a repressed (denoted *Pl′*) state. At least sixteen loci whose functions are *required to maintain repression (rmr)* of *Pl′* have been identified in an ethyl methanesulfonate mutagenesis screen. Three of the four known RMR proteins that mediate 24 nucleotide (24nt) RNA biogenesis are putative orthologs of *Arabidopsis* proteins central to the RNA-directed DNA Methylation (RdDM) pathway which directs repressive chromatin modifications. The fourth protein is unique to 24nt RNA biogenesis in maize. Here we describe three recessive alleles which define the *rmr12* locus and complement previously identified *rmr* factors. Unlike any other *rmr*-type mutations found to date[2, 3, 4, 5, 6], *rmr12* mutants display a unique combination of defects, including male gametophyte dysfunction, that indicate a novel mechanistic connection between paramutation and developmental gene control. Whole genome sequence analysis and molecular mapping place *rmr12* in an interval on chromosome *9S* containing 109 gene models; none of which have annotated functions consistent with known RdDM-type proteins. The molecular identification of *rmr12* promises to highlight a novel component of an epigenetic system specifying both heritable changes in gene regulation and proper ontogeny.

Citations: **1**. Hollick *et al*. 1995 *Genetics* **141**, 709. | **2**. Dorweiler *et al*. 2000 *Plant Cell* **20**, 2101. | **3**. Parkinson *et al*. 2007 *Dev. Biol*. **308**, 462. | **4**. Hale *et al*. 2007 *PLoS Biol*. **5**, 2156 | **5**. Stonaker *et al*. 2009 *PLoS Genet*. **5**, e1000706. | **6**. Barbour *et al*. 2012 *Plant Cell* **24**, 1761

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P237

The role of boron in vegetative and reproductive development in maize

(submitted by Michaela Matthes <matthesm@missouri.edu>)
Full Author List: Matthes, Michaela[1]; Durbak, Amanda[1]; McSteen, Paula[1]
[1] Bond Life Sciences Center, Department of Biological Sciences, University of Missouri-Columbia, Columbia, MO 65211

Boron is an essential micronutrient whose deficiency is widespread in the US and worldwide causing yield decline in many crops. We have identified the *tassel-less1* (*tls1*) mutant in maize, which is inherently boron deficient, due to its impaired uptake of boron out of the soil. The mutation particularly effects meristematic tissues, leading to vegetative and/or reproductive defects depending upon the boron concentration in the soil. In low boron soils, *tls1* shows severe leaf defects and dies at the seedling stage, whereas in higher boron soils, the mutant has a reduced or no tassel as well as a reduced or no ear. Although boron plays a well described stabilizing role in the cell wall, we have experimental evidence for additional roles of boron beyond the cell wall. We are currently analyzing the interaction between boron and the hormone pathways regulated by auxin or cytokinin using double mutant analysis and confocal microscopy with marker genes. Additionally, we are determining the time points when the first defects of boron deficiency can be observed, in vegetative as well as tassel and ear meristems using time course and boron supplementation experiments combined with SEM and histology. Our studies will aid in understanding the mechanisms regulating plant responses to boron deficiency. This is critical for improving crop tolerance to this environmental stress and will eventually lead to the development of high-yielding plants with optimized growth in marginal soils.

Funding acknowledgement: United States Department of Agriculture (USDA)

P238

The role of CT2 in maize meristem development

(submitted by Dave Stateczny <dave.stateczny@uni-hamburg.de>)
Full Author List: Stateczny, Dave[1]; Blaha, Andreas[1]; Kluth, Jantjeline[1]; Oppenheimer, Jara[1]; Bommert, Peter[1]
[1] University of Hamburg, Developmental Biology, Hamburg, 22609, Germany

Heterotrimeric G proteins are membrane-associated molecular switches involved in the transduction of extracellular signals to induce specific cellular responses by activating downstream effectors. They are composed of the three subunits Gα, -β and -γ. We identified COMPACT PLANT2 (CT2) as the maize Gα-subunit. *ct2* mutants exhibit cell proliferation defects resulting in a severely altered plant architecture. Our analysis of a functional CT2-YFP reporter line reveals high CT2-YFP expression levels located to the developing phragmoplast, indicating a novel function of G protein signaling in cytokinesis.
G protein signaling in plants also differs in its regulation as the Gα subunit exhibits unusual GTP hydrolysis kinetics. The combination of spontaneous GTP-loading and low GTPase activity suggests that Gα subunits in plants are bound to GTP by default, and are thus proposed to be constitutively active. Another important difference with regard to animal systems is that plants do not have functional GPCRs. These findings have profound implications for the mechanisms of G protein regulation and it has been proposed that regulation of G protein signaling activity in plants is modulated via the deactivation of Gα activity through GTPase accelerating proteins (GAPs). In *Arabidopsis* it has been shown that the phospholipase AtPLDα1 physically interacts with Gα to accelerate GTP hydrolysis *in vitro*, indicating that AtPLDα1 has GAP activity. We identified the maize PHOSPHOLIPASE Dα5 (PLDα5) in our initial CT2-YFP IP/MS experiments. This interaction might expand the regulatory network of maize G protein signaling. We are currently validating this interaction by pursuing yeast-2-hybrid assays and FRET analysis *in vivo*.

Funding acknowledgement: German Science Foundation

P239

The role of *Suppressor of sessile spikelet1 (Sos1)* in important meristem maintenance pathways in maize

(submitted by Eden Johnson <ejcv4@mail.missouri.edu>)
Full Author List: Johnson, Eden A[1]; Wu, Xinting[1]; Kellogg, Elizabeth[2]; McSteen, Paula[1]
[1] Division of Biological Sciences, University of Missouri, Columbia, MO 65202
[2] Donald Danforth Plant Sciences Center, St. Louis, MO 63132

Meristems control organogenesis in plants in part through the maintenance of groups of undifferentiated stem cells. Upon completion of vegetative development, the shoot apical meristem is converted into the inflorescence (or "flowering branch") meristem. Inflorescences of grass species (Poaceae) bear spikelets, which house the male and female floral organs and are the fundamental units of grass inflorescence architecture. Although solitary spikelets are shared by several major agro-economic crops (e.g., wheat, rice, and barley), paired spikelets are produced by species in at least three grass tribes (e.g., Paniceae, Paspaleae, and Andropogoneae), including maize. Three loci have been identified in the *Suppressor of sessile spikelet (Sos)* class of mutants in maize which regulate the production of paired spikelets. Sos1 is a semi-dominant mutant that prevents the formation of the sessile spikelet, causing only single spikelets to form. Sos1 also has developmental defects in the inflorescence meristem indicating additional roles in development. Genetic interaction analyses and scanning electron microscopy (SEM) are being used to characterize the interaction with other maize meristem mutants, namely *thick tassel dwarf1, fasciated ear2,* and *compact plant2*. Results suggest that Sos1 may be a player in key meristem maintenance pathways. In a parallel study, we are using SEM and catalogued herbarium specimens to determine the developmental ontogeny of selected grasses with single versus paired spikelets in the Paniceae tribe. Our long term goal is to integrate the molecular, genetic, and developmental differences between species with paired or solitary spikelets in order to unravel the mechanisms behind the development of this agronomically important trait.

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P240

The transcriptional co-repressor REL2 regulates meristem initiation, determinacy and maintenance in maize inflorescences

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

Transcriptional repression is an essential and widespread mechanism regulating plant development pathways, hormone signal transduction, circadian rhythms and various responses to biotic and abiotic stresses. The maize RAMOSA1 ENHANCER LOCUS2 (REL2) protein belongs to a small, highly conserved family of transcriptional co-repressors that are present in mosses and all higher plants, whose founding member is the Arabidopsis TOPLESS (TPL) protein. TPL proteins do not bind DNA directly, but are recruited by specific transcription factors and other transcriptional adaptor proteins to inhibit transcription of diverse downstream target genes.
Recessive rel2 mutants display striking vegetative and reproductive defects that are particularly severe in standard inbred backgrounds. In particular, rel2 tassels have fewer branches and spikelets, while ears (when formed) are slightly fasciated, suggesting that REL2 functions in meristem initiation and maintenance pathways. The array of phenotypes observed in rel2 mutants suggests that REL2-mediated repression plays a fundamental role in regulating the transcriptional outputs of many pathways throughout maize vegetative and reproductive development. To investigate pathways that use REL2 to control transcriptional outputs, we performed a series of genetic, genomic and molecular experiments. We have identified more than 70 transcription factors that interact with REL2 proteins. Among these are two key transcriptional regulators of spikelet development, IDS1 and SID1, which together control meristem determinacy and floral organ identity. Analysis of rel2, ids1 and sid1 double and triple mutants suggest that IDS1 and SID1 directly repress their target genes by REL2-mediated transcriptional repression. Parallels between floral development in monocotyledonous and dicotyledonous species will be presented.

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P241

Towards live imaging of meiosis in maize

(submitted by Martina Balboni <martina.balboni@uni-hamburg.de>)
Full Author List: Balboni, Martina[1]; Prusicki, Maria Ada[1]; Schnittger, Arp[1]
[1] University of Hamburg - Biozentrum Klein Flottbek; Department of Developmental Biology; Hamburg, Ohnhorststrasse 18 - 22609 - Germany

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, e.g. how homologous chromosomes recognize each other. 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 is based on transgenic lines producing fluorescently labeled proteins that highlight hallmarks of meiosis, e.g. chromosome synapsis, cohesion, etc. A first aim of this method is to establish a detailed time course of meiotic recombination and progression in maize.

P242

Trait-first, models for yield improvement in crops

(submitted by Alexander Goldshmidt <agold@monsanto.com>)
Full Author List: Goldshmidt, Alexander[1]; Preuss, Sasha[2]
[1] 37437 State Highway 16, Woodland CA 95695
[2] 700 Chesterfield park way, Chesterfield MO,63017

Climate change, population growth, and decline in arable land are just a few among multiple factors threatening adequate food and raw materials supply for the growing world population. To maintain and improve availability of the basic supplies to the society while preserving and expanding remaining wild life resources, technological advancement in agriculture is essential. Particularly important is to increase crop productivity per unit area-yield. Yield performance of the major agricultural crops could be improved through comprehensive approach involving different aspects of agriculture such as agronomy, use of biologicals, microbial, breeding and biotech. Scientific advancements of the last twenty years in plant biology significantly improved our ability to improve field crop traits by modifying gene expression and activity with biotechnology tools. While these improvements have been important, attempts to boost yield performance via manipulation of the single morphological, physiological or developmental traits had only limited success so far. For this reason, future use of the biotech and gene editing tools for yield improvement requires expanded understanding of the trait to yield relationship. In current work, we use spring wheat as an experimental model to examine relationship between inflorescence morphology, maturity and yield. We show that highest yield outcome is more likely to be achieved via optimization of trait by trait equilibrium rather then by strong modification of the single trait.

Funding acknowledgement: Monsanto

P243

Transcriptomic analyses of Leaf Cuticular-Epidermal development in maize

(submitted by Pengfei Qiao <pq26@cornell.edu>)
Full Author List: Qiao, Pengfei[1]; Molina, Isabel[2]; Bourgault, Richard[2]; Smith, Laurie[3]; Gore, Michael[4]; Scanlon, Michael J.[1]
[1] Division of Plant Biology, Cornell University, Ithaca, New York 14853, USA
[2] Biology Department, Algoma University, Sault Ste. Marie, ON P6A 2G4, Canada
[3] Division of Biological Sciences, UC San Diego, La Jolla, CA 92093, USA
[4] Division of Plant Breeding and Genetics, Cornell University, Ithaca, New York 14853, USA

Cuticles serve important roles in protecting plants from water loss and pathogen attack. Composed of cutin and epicuticular waxes, cuticles are deposited on the epidermis of the plant shoot. We have performed biochemical profiling along a gradient in the developing adult leaf in maize. Both wax and cutin show significant differences along the proximal-distal axis of the expanding eighth leaf. To date, no transcriptomic study has explored the differences in gene expression in the epidermis of the expanding, adult maize leaf. In this study, we laser-microdissected the epidermis and non-epidermal tissues of leaf 8 in the inbred B73. After linear amplification of the tissue-specific RNAs, Illumina based RNA-seq identified transcripts implicated in cuticle development in maize. Differentially expressed genes were identified, and K means and hierarchical clustering unravels the lipid biosynthesis genes and transcription factors correlated with the cuticlar difference along the leaf. This study provides a transcriptomic perspective on cuticle development that is applicable to genetic strategies for drought tolerance in maize.

Funding acknowledgement: National Science Foundation (NSF)

P244

Uncovering developmental intermediates in pre-meiotic anther development using single-cell RNA-seq

(submitted by Bradlee Nelms <bnelms.research@gmail.com>)
Full Author List: Nelms, Bradlee[1]; Walbot, Virginia[1]
[1] Stanford University; 385 Serra Mall Rd; Stanford, CA, 94305

In maize anthers, archesporial (AR) cells are the first cell type committed to meiosis and pollen production. During the ~7 day window between when AR cells first arise in the anther primordia and when these cells differentiate into pollen mother cells (PMCs), the surrounding somatic tissue goes through several major developmental transitions. In contrast, it is unclear to what extent the AR cell population itself is changing, due to the lack of obvious morphological variation in the AR cells during this time period (other than an increase in cell number and size). We are testing the hypothesis that AR cells go through discrete developmental stages as they mature into PMCs and prepare for meiosis using single-cell RNA-Seq. Single-cell RNA-Seq can distinguish distinct classes of cells without the need for known marker genes, providing a valuable opportunity to dissect this poorly understood phase of anther development.

Funding acknowledgement: National Science Foundation (NSF)

P245

Using *Setaria viridis* to accelerate the characterization of candidate genes in kranz anatomy development

(submitted by Carla Coelho <ccoelho@danforthcenter.org>)
Full Author List: Coelho, Carla P.[1]; Van Eck, Joyce[2]; Pidgeon, Kaitlin[2]; Mayfield-Jones, Dustin[1]; Baca, Matthew[1]; Gil-Humanes, Javier[3]; Voytas, Dan[3]; Evans, Bradley[1]; Berg, Howard[1]; Brutnell, Thomas P.[1]
[1] Donald Danforth Plant Science Center, Saint Louis, MO 63132, USA
[2] Boyce Thompson Institute, Ithaca, New York 14853, USA
[3] Department of Genetics, Cell Biology, and Development, and Center for Genome Engineering, University of Minnesota, Minneapolis, MN 55455, USA

C_4 photosynthesis relies on both biochemical and anatomical adaptations; and while the biochemistry is well established, the regulatory networks underlying Kranz anatomy are largely unknown. Recently, a number of candidate genes underlying Kranz anatomy have been identified including a regulatory network involving the transcriptional module of SHORT-ROOT/SCARECROW (SHR/SCR). The interactions of SHR/SCR with IDDs have been hypothesized to determine cell identity in the leaves of C_4 species. The complexity of these gene networks and time to generate mutations in several genes in maize has hampered functional analyses of these candidates. We have thus pioneered the use of *Setaria viridis* as a model C_4 grass to dissect the function of genes involved in the establishment of Kranz anatomy. We have successfully applied CRISPR/Cas9 technologies to generate null alleles in *S. viridis*. In parallel, translational fusion constructs tagged with YPet were developed for cellular localization and ChIPseq. Confocal and light microscopy in single and higher order mutants is being used to evaluate defects in cellular patterning or differentiation of the vascular bundle in the independently evolved *Setaria viridis* and also in maize. We hypothesize that BS- or M-cells enriched IDDs act together with SHR and SCR define a network that contributes to the differentiation of photosynthetic cells in C_4 grasses.

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P246

ZmDof3, a maize endosperm-specific Dof protein gene, regulates starch accumulation and aleurone development in maize endosperm

(submitted by Jingjuan Yu <yujj@cau.edu.cn>)
Full Author List: Qi, Xin[1]; Yu, Jingjuan[1]
[1] College of Biological Sciences, China Agricultural University,No2.Yuanmingyuan West Road ,Beijing 100193, China

To explore the function of Dof transcription factors during kernel development in maize, we first identified Dof genes in the maize genome. We found that *ZmDof3* was exclusively expressed in the endosperm of maize kernel and had the features of a Dof transcription factor. Suppression of *ZmDof3* resulted in a defective kernel phenotype with reduced starch content and a partially patchy aleurone layer. The expression levels of starch synthesis-related genes and aleurone differentiation-associated genes were down-regulated in *ZmDof3* knockdown kernels, indicating that ZmDof3 plays an important role in maize endosperm development.

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P247

ZmNST3 and ZmNST4 are master switches for secondary wall deposition in maize (*Zea mays* L.)

(submitted by Jingjuan Yu <yujj@cau.edu.cn>)
Full Author List: Xiao, Wenhan[1]; Yu, Jingjuan[1]
[1] State Key Laboratory for Agro-biotechnology, College of Biological Sciences, China Agricultural University,No2.Yuanmingyuan West Road ,Beijing 100193, China

Secondary walls are the most abundant biomass produced by plants, and they consist mainly of lignin, cellulose and hemicellulose. Understanding how secondary wall biosynthesis is regulated could potentially provide genetic tools for engineering biomass components, especially in maize and Sorghum bicolor. Although many works have focused on secondary wall biosynthesis in dicotyledons, little has been reported for these monocotyledons. In this study, we cloned two NAC transcriptional factor genes, *ZmNST3* and *ZmNST4*, and analyzed their functions in maize secondary wall formation process. ZmNST3 and ZmNST4 were expressed specifically in secondary wall-forming cells, expression of *ZmNST3/4* can restore the pendent phenotype of Arabidopsis nst1nst3 double mutant. *ZmNST3/4*-overexpressing Arabidopsis and maize displayed a thickened secondary wall in the stem, and knockdown maize showed defective secondary wall deposition. ZmNST3/4 could regulate the expression of *ZmMYB109/128/149*. Our results revealed that ZmNST3/4 are master switches of the maize secondary wall biosynthesis process and provides new evidence that the secondary wall regulatory pathway is conserved in different plant species.

Funding acknowledgement: National Basic Research Program of China

P248

Zygotic genome activation occurs shortly after fertilization in maize

(submitted by Thomas Dresselhaus <thomas.dresselhaus@ur.de>)
Full Author List: Chen, Junyi[1]; Strieder, Nicholas[2]; Krohn, Nadia G.[3]; Cyprys, Philipp[1]; Sprunck, Stefanie[1]; Engelmann, Julia C.[2]; Dresselhaus, Thomas[1]
[1] Cell Biology and Plant Biochemistry, Biochemie-Zentrum Regensburg, University of Regensburg, 93053 Regensburg, Germany
[2] Institute of Functional Genomics, University of Regensburg, 93053 Regensburg, Germany
[3] Department of Agriculture, Regional Campus of Umuarama, State University of Maringa, 87507-190, Umuarama, PR, Brazil

The formation of a zygote by the fusion of an egg and a sperm cell and its subsequent asymmetric division (ACD) represent the first hallmarks at the beginning of the plant life cycle. In animals, it was shown that the initial steps of zygote and embryo development are mainly maternally controlled and zygotic genome activation (ZGA) is delayed. In plants the timing of ZGA is not known due to technical difficulties to access the deeply embedded egg cells and zygotes. Using maize as a plant model system we have determined the timing of zygote development in vivo. We manually isolated living cells at different stages and generated RNA-seq transcript profiles of gametes, zygotes as well as its apical and basal daughter cells after ACD. We report that ZGA occurs shortly after fertilization in maize with >10% of the whole genome being activated. Compared with the cell cycle especially genes encoding transcriptional regulators of various families are quickly activated in the early zygote. Overall the zygotic transcriptome is highly dynamic and ZGA appears to occur in an all at once-reaction. Moreover, we report the identification of large numbers of genes with restricted expression pattern with associated functions, for example, in chromatin assembly and transcriptional activity, fertilization, ACD, cell fate identity, hormone responses and various signalling pathways.

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P249

EMB15 functions in plastid 30S ribosome assembly and embryogenesis in maize

(submitted by Chunhui Xu <chunhuixu@sdu.edu.cn>)
Full Author List: Xu, Chunhui[1]; Shen, Yun[1]; Li, Cuiling[1]; Lu, Fan[1]; Meeley, Robert[2]; McCarty, Donald R.[3]; Tan, Bao-Cai[1]
[1] Key Laboratory of Plant Cell Engineering and Germplasm Innovation, Ministry of Education, School of Life Sciences, Shandong University, Jinan, China
[2] DuPont Pioneer AgBiotech Research, Johnston, Iowa 50131-1004, USA
[3] Horticultural Sciences Department, University of Florida, Gainesville, FL 32611, USA

The embryo defective (*emb*) mutants account for a major group of seed mutants in maize, which display specifically arrested embryogenesis but nearly normal endosperm development. We characterized the *Emb15* mutant in maize with the cloning of the causal gene. The *emb15* mutants displayed an emb phenotype in the W22 background. *Emb15* encodes a unique protein with an N-terminus domain homologous to prokaryotic RimM proteins and a C-terminus domain similar to UDP-GlcNAc pyrophosphorylases (UAP). Thus, *Emb15* appears to be derived from a fusion of two genes as moss and lower species host the two domains in two separate proteins. The RimM protein in *Escherichia coli* is implicated in the assembly of 30S ribosome and is essential for growth, whereas UAP is considered to catalyze a reversible reaction converting UTP and GlcNAc to UDPGlcNAc and PPi, the precursor of N- and O-linked glycosylation. EMB15 is localized in the chloroplast and nucleus. Expression of *Emb15* in ΔrimM mutant in *E. coli* partially restores the growth. Y2H analysis indicated that EMB15 interacts with chloroplast ribosomal protein S19, which is the same partner of RimM in *E. coli*. These results suggest that EMB15 may have a similar function of RimM in facilitating 30S ribosome assembly in chloroplasts. The function of UAP domain is under study.

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P250

PPR24 functions in the C-to-U editing of mitochondrial *nad7* introns that is essential for intron splicing and complex I assembly in maize

(submitted by Chunhui Xu <chunhuixu@sdu.edu.cn>)
Full Author List: Xu, Chunhui[1]; Song, Shu[1]; Yang, Yanzhuo[1]; Lu, Fan[1]; Tan, Bao-Cai[1]
[1] Key Laboratory of Plant Cell Engineering and Germplasm Innovation, Ministry of Education, School of Life Sciences, Shandong University, Jinan, China 250199

Pentatricopeptide repeat (PPR) proteins are implicated in organellar RNA metabolism that is important to plant growth and development. Despite their importance, the functions of most PPR proteins remain unknown. Here we report the determination of the function of PPR24 and its role in growth and development in maize. PPR24 is a PPR-DYW subgroup protein with 10 PPR motifs and a DYW domain. The loss of function mutants displayed arrested embryo and endosperm development, giving rise to a small and defective kernel phenotype. Subcellular localization revealed that PPR24 is targeted to mitochondria. BN-PAGE analysis indicated that the assembly of mitochondrial complex I is disrupted and the NADH dehydrogenase activity is lost in the *ppr24* mutant. However, editing analysis of the mitochondrial coding sequences did not find defects accountable for the severe phenotype. Surprisingly, analyses of the transcripts revealed that the splicing of *nad7* intron 3 and 4 are severely reduced in the *ppr24* mutant, raising the possibility that PPR24 may function either in the splicing of the two introns or in the editing of the introns and such editing is critical for the splicing. Comparison of the spliced introns between the wild-type and *ppr24* mutant revealed that the mutant is completely deficient in the C-to-U editing of *nad7*-intron3-878 and *nad7*-intron4-1581 which are completely edited in the wild-type. Intron modeling suggested that both sites are located in domain V of the introns and the loss of editing could alter the structure of this domain, providing a possible scenario that the deficiency in *nad7* intron 3 and 4 splicing in *ppr24* is due to loss of editing at the two sites. Hence PPR24 may function in the editing of two sites that are essential for the intron splicing. The deficiency in splicing impairs complex I assembly and mitochondrial function, leading to defective seed development.

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P251

A cohesin subunit may facilitate homologous chromosome pairing in meiotic prophase in maize

(submitted by Jing Zhang <zhangjing@genetics.ac.cn>)
Full Author List: Zhang, Jing[1]; Birchler, James A.[2]; Han, Fangpu[1]
[1] State Key Laboratory of Plant Cell and Chromosome Engineering, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing, 100101, China
[2] Division of Biological Sciences, 311 Tucker Hall, University of Missouri, Columbia, Missouri, 65211, USA

Meiosis is a highly conserved eukaryotic cell division by which sexual reproduction maintains the species-specific chromosome number across generation. Successful completion of meiosis depends on homologous chromosome pairing, a requirement to establish physical association between homologs. Recent research in many organisms indicated that centromere interactions during early meiotic prophase facilitate homologous chromosome pairing, and a functional centromere is a prerequisite for centromere pairing as shown in maize. We used ChIP-MS analysis of histone H3 variant CENH3-associated factors immunoprecipitated from early meiotic prophase anthers to identify candidate proteins that can regulate the centromere pairing process in maize. Structural maintenance of chromosome 3 (SMC3) was identified to interact with CENH3 at the leptotene stage when homologous chromosomes begin to pair. Immunostaining results showed that SMC3 is located on chromosomes and can be detected in the centromeric region of all chromosomes from leptotene to the pachytene stage. In somatic cells, SMC3 was associated with chromatin from prophase to anaphase. In the *afd1-1* mutant, which exhibits absence of centromere pairing in early meiotic prophase I, SMC3 is not detectable from leptotene to pachytene. In the *phs1* mutant, centromere pairing is incomplete in early meiotic prophase and SMC3 exhibits very weak signals compared to the wild type. We used CRISPR-Cas9 technology to generate transgenic maize plants that carry mutations in the *ZmSMC3* gene, *smc3* mutants showed premature loss of sister chromatid cohesion in mitotic spreads. These data suggest SMC3 may have multiple functions in maize.

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P252

A transgenic Double Ds chromosome breaking system in maize

(submitted by James Birchler <birchlerj@missouri.edu>)
Full Author List: Krishnaswamy, Lakshminarasimhan[1]; Zhao, Changzeng[1]; Albert, Patrice[1]; Torno, Alessandra[1]; Nastasi, Louis[1]; Gao, Zhi[1]; Birchler, James[1]
[1] University of Missouri, Columbia, Missouri 65211

A transgenic Double Ds system for chromosome breakage was developed. An Agrobacterium construct was assembled that contains a Bar selection marker and a Ubiquitin promoter-loxP-B-peru reporter between the left and right borders of a T-DNA. The Ubiquitin promoter-bar gene is surrounded by the terminal inverted repeats of Dissociation (Ds) except that they are oriented in a direct relationship, which has previously been shown to cause chromosomal breakage when acted upon by Activator (Ac). Fifty transformation events have been recovered. Fluorescence in situ hybridization for the transgene was used to localize several to chromosome arm to date. One that is localized proximally on chromosome arm 4S was crossed to a stable Activator insertion on 1S, stAc5145, and then self-pollinated with subsequent screening for homozygotes of the transgene and the stable Ac element. When these homozygotes were crossed as males onto a sugary tester, a fine pitting of the endosperm was observed, consistent with chromosomal breakage in 4S to uncover sugary in the presence of Ac. No such phenotype is observed in the absence of Ac. When these materials were crossed as males onto bt2/+ plants, segregation was found for mosaic brittle sectors, again consistent with chromosome breakage in 4S. Another insertion event is present in the B chromosome. The transgene expresses B-peru that in the appropriate genetic background conditions anthocyanin color in many tissues. This event provides a phenotypic marker to an otherwise normal B chromosome that exhibits the nondisjunction property. These transgene insertions provide the potential to direct chromosomal breakage at defined sites (~50) in the genome. The transgene carries a loxP site that could potentially be targeted for site-specific integration, which would simultaneously disrupt the expression of the B-peru gene. This system could also be useful for mosaic developmental and genetic autonomy analyses and the mosaic loss of maternal chromosomes in the endosperm for studies of parental gene imprinting among other applications. Research supported by NSF grant IOS-1339198.

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P253

Assessment of the diversity of the abnormal chromosome 10 meiotic drive system in *Zea mays*

(submitted by David Higgins <dmhiggin@uga.edu>)
Full Author List: Higgins, David M.[1]; Dawe, R. Kelly[1 2]
[1] Department of Plant Biology, University of Georgia, Athens, GA, USA 30602
[2] Department of Genetics, University of Georgia, Athens, GA, USA 30602

Meiotic drive is the preferential inheritance of a selfish genetic element at a rate greater than Mendelian expectations. Abnormal chromosome 10 (Ab10) in *Zea mays* (maize) is a variant of normal chromosome 10 (N10) which exhibits meiotic drive, being inherited at rates of approximately 70% from offspring of Ab10/N10 females. Despite this strong driving pressure, Ab10 is only seen at low frequencies in maize landraces, indicating that there must be a negative consequence of inheriting this version of chromosome 10. In order to better understand how the drive mechanism works and what factors may be limiting it, a series of experiments were carried out on a diversity panel of different accessions of Ab10. We found no evidence that Ab10 causes defects in development of the male gametophyte, though pollen viability is significantly lower in Ab10 homozygotes. An enrichment of variants were identified in the inverted shared region an Ab10 haplotype. Rates of drive are largely consistent across Ab10 haplotypes and phylogenetic analysis suggests exchange of genes between them. This study represents the first analysis of different Ab10 haplotypes at the transcriptome level.

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P254

B chromosome contains active genes and impacts the transcription of A chromosomes in Maize (Zea mays L.)

(submitted by Wei Huang <wilsonhuang23@cau.edu.cn>)
Full Author List: Huang, Wei[1]; Du, Yan[1]; Zhao, Xin[1]; Jin, Weiwei[1]
[1] National Maize Improvement Center of China, Beijing Key Laboratory of Crop Genetic Improvement, China Agricultural University, Beijing 100193, China

The dispensable maize (Zea mays L.) B chromosome is highly heterochromatic and widely believed to be devoid of functional genes. Although low-copy B chromosome causes no obvious phenotype variation, its existence might influence A genome gene expression. Previous studies found several maize B chromosome derived transcripts, but it is still not clear whether these transcripts are part of protein-coding genes. Moreover, up to now, few discernible genes have been revealed on the B chromosomes in maize. In our study, we applied RNA-seq and cytological analysis to expand our knowledge about maize B chromosome transcription and its impacts on maize transcriptome. Our data suggested that maize B chromosome influences the A-genome transcription with stronger effect associated with an increase in copy number of B chromosome. In total 130 differently expressed genes were detected in comparison between with and without B chromosome lines. These differentially expressed genes are mainly involved in cell metabolism and nucleotide binding. Using Starter+B, we amplified ten B chromosome loci with high sequence similarity to A-genome genes. Fluorescence in situ hybridization (FISH) confirmed that at least four ~5kb-sized genes are located on the B chromosome. In addition, through de novo assembly of the reads not unmapped to maize B73 reference genome together with PCR validation, we found three B-located LTR; in particular, one of them, the 3.2 kb comp75688, is expressed in a B-dosage dependent manner. Collectively, we found that in the presence of maize B chromosome, the transcription of A genome genes was altered, with more impact by the increase of the B chromosome number. The B-located transcriptionally active genes showed high similarity to their A-genome homologues, and retrotransposons on B chromosome also have partial homologous to A genome sequences. Our data shed more lights on the genome structure and evolution of the maize B chromosome.

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P255

Chromatin landscape of meiotic recombination in maize

(submitted by Adele Zhou <az266@cornell.edu>)
Full Author List: Zhou, Adele[1]; Sidhu, Gaganpreet[2]; He, Yan[3]; Wang, Minghui[1]; Dukowic-Schulze, Stefanie[4]; Avoles-Kianian, Penny[4]; Pawlowski, Wojtek[1]
[1] Cornell University; Ithaca, NY, 14853
[2] Columbia University Medical Center; New York, NY, 10032
[3] China Agricultural University; Beijing, China, 100083
[4] Univeristy of Minnesota; Minneapolis, MN, 55455

Meiotic recombination is initiated by programed double-strand breaks (DSBs) in chromosomal DNA and results in crossovers (COs) and non-crossovers (NCOs). In many organisms, there is an excessive number of meiotic DSBs to the number of COs, but it is not well understood how this process is regulated and which DSBs become COs. In the B73 inbred of maize, ~500 DSBs are observed in mid zygotene which lead to ~20 COs. These DSBs are evenly distributed along chromosomes including pericentromeric and centromeric regions. However, CO distribution does not follow the DSB distribution. COs in B73 are enriched in distal chromosome regions that are known to be euchromatic. COs are suppressed in proximal regions containing the pericentromeric and centromeric regions, which are generally heterochromatic. In the CML228 inbred of maize, there are only ~200 observed DSBs, which is roughly half the number of DSBs in B73. These DSBs result in only 11 COs. Unlike COs in B73, CML228 COs are located in the heterochromatic proximal regions of the chromosome. We are using these two maize inbreds with diverse DSBs and CO distributions to investigate the dynamics of recombination events in euchromatic and heterochromatic chromosomal regions and to understand how this distribution is formed. Results of our experiments indicate that the occurrence of DSB events in euchromatic and heterochromatic chromosomal regions do not differ significantly between these two inbreds. Instead, they can be attributed to processes of DSB repair which occur in later stages of meiotic prophase I.

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P256

Fast-flowering mini-maize: seed to seed in 60 days update

(submitted by Morgan McCaw <mem7b6@mail.missouri.edu>)
Full Author List: McCaw, Morgan E.[1]; Birchler, James A.[1]
[1] Division of Biological Sciences; University of Missouri; Columbia, MO, 65211

Fast-Flowering Mini-Maize (FFMM) are two recently developed, miniature lines of maize selected to improve upon maize as a model organism. Maize has many characteristics which make it a good model, such as separate male and female flowers, large number of progeny, self-compatibility, and a long history as a research model during which many techniques for study have been developed. The typical maize line can produce about three generations per year using a greenhouse, while FFMM can produce five generations per year regularly, though six generations may be possible with optimized conditions. FFMM plants can also be grown with smaller pots and take up less space than standard maize plants. An article describing the attributes of FFMM was recently published, which included an Illumina sequence for the "A" line aligned to the B73 reference genome. Seed for lines A and B, as well as *R-scm2* and *y1* color marker introgressions into FFMM-A are available through the Maize Genetics Coop. Current work with FFMM is focusing on introgression and selection of factors for Type II callus formation under tissue culture into FFMM-A.

P257

Hijacking a quirk of Stock-6 based haploid inducer lines to rapidly transfer B chromosomes and minichromosomes to multiple isogenic lines

(submitted by Morgan McCaw <mem7b6@mail.missouri.edu>)
Full Author List: McCaw, Morgan E.[1]; Birchler, James A.[1]
[1] Division of Biological Sciences; University of Missouri; Columbia, MO, 65211

B chromosomes are supernumerary chromosomes which have an active centromere, but no known vital genes. B chromosomes can be truncated with a construct containing genes of interest and a telomere repeat to create a minichromosome. This minichromosome does not recombine with normal maize chromosomes, which eliminates the complications of linkage drag with the transgene. The inclusion of site-specific recombination sites in the transgene can allow for subsequent stacking of additional genes on the minichromosome.
Stock 6 based haploid inducer (HI) lines produce maternal haploids at a rate of ~1-8% when used as the male parent in a cross. These lines are ubiquitous in major breeding programs because the maternal haploids can be doubled in ploidy, then self-pollinated to produce a completely isogenic line. Rarely, the transfer of portions of the paternal genome to otherwise maternal haploids has been noted. Elevated rates of kernel and embryo abortion, and some unusual phenotypes in putative haploids may indicate aneuploidy resultant from the transfer of an incomplete complement of the paternal chromosomes.
We have attempted to transfer whole B chromosomes and minichromosomes by back-crossing them into an HI line, then used those plants carrying a minichromosome or B chromosome to produce haploids. The resultant haploids are screened for the extra chromosomes. This method could simplify breeding programs which involve transgenes by moving them as a single unit to multiple inbred lines in just two generations (the induction cross then a doubling and selfing of the haploid) after introgression into the HI line. B chromosomes have been successfully transferred to multiple inbred lines using this method. Additionally, aneuploid progeny with 1N+1 and 2N-1, as well as a rearrangement of a transferred B chromosome have been identified.

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P258

Maize by Monet: Developing whole chromosome paints

(submitted by Patrice Albert <albertp@missouri.edu>)
Full Author List: Albert, Patrice S.[1]; Zhang, Tao[2 3]; Jiang, Jiming[2]; Birchler, James A.[1]
[1] Division of Biological Sciences, University of Missouri, Columbia, Missouri, USA 65211
[2] Department of Horticulture, University of Wisconsin, Madison, Wisconsin, USA 53706
[3] College of Agriculture, Yangzhou University, Yangzhou, Jiangsu Province, P. R. China 225009

The goal of the whole chromosome paint project is to fluorescently label each pair of the ten maize chromosomes a unique color. The paints can be used as a tool to visually study chromosomal rearrangements, aneuploid events, the phylogeny of related species, and chromosome pairing. Our previous work showed proof of concept but for long-term utility, the methods used were either time-prohibitive (manual selection of non-repetitive sequences, labeling the sequences individually) or cost-prohibitive (limited amount of probe manufactured by a third party), even when painting only two or three chromosomes. Recent developments in oligonucleotide design and the ability to amplify them have allowed the project to advance.
Non-repetitive sequence selection is computationally determined. The oligo synthesis and amplification methods produce an extensive collection of DNA oligos that can be used to make probes. Additionally, all sequences for any given chromosome can be labeled simultaneously and with a fluorochrome chosen by the researcher to suit the needs of the experiment. Thus far, eight of the maize chromosome oligo libraries have been quality checked for chromosome specificity using fluorescence *in situ* hybridization methods. Work is underway to select combinations of fluorochromes that, when used to label the same sequences, will produce easily distinguished, unique colors that are distinct for each of the ten chromosomes. Funded by NSF grant IOS-1444514.

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P259

Numerous chromosomal variants derived from B chromosome irradiation

(submitted by Yalin Liu <ylliu@genetics.ac.cn>)
Full Author List: Liu, Yalin[1 2]; Su, Handong[1 2]; Zhang, Jing[1]; Liu, Yang[1 2]; Gao, Zhi[3]; Birchler, James. A[3]; Han, Fangpu[1]
[1] State Key Laboratory of Plant Cell and Chromosome Engineering, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing, China 100101
[2] University of Chinese Academy of Sciences, Beijing, China 100049
[3] Division of Biological Sciences, University of Missouri, Columbia, Missouri 65211-7400

The B chromosome is supernumerary chromosome that is dispensable. The exact origin of B chromosome is not clear. In maize, some B-A translocation chromosomes were useful for the study of the process of de novo centromere formation and change of centromere activity. With the B chromosome sequence nearing completion, B chromosome irradiation was performed to study the role of various B regions. During the screening of the irradiated offspring, several chromosomal variants have been found. The most common cases are the mini fragments containing an active B centromere region with different sizes, which can be used to refine the sites required for B centromere nondisjunction. Another common result is B-A reciprocal translocation chromosomes with random B chromosome breakpoints. Also found was a series of dicentric chromosomes, which can be used to examine centromere inactivation. There are also derivatives of the B chromosome with different deletions on the long arm, which can aid to refine the precise location of nondisjunction sites, especially the one in the proximal euchromatin. These numerous B chromosome variants together with the B chromosome sequence will aid in the eventually elucidation of the various unusual behaviors of the B chromosome.

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P260

Phosphorylation of histone H3 Thr3 by Haspin is correlated with cohesion during the cell cycle

(submitted by Yang Liu <yangliu@genetics.ac.cn>)
Full Author List: liu, yang[1 2]; su, handong[1 2]; liu, yalin[1 2]; zhang, jing[1]; Birchler, James A[3]; han, fangpu[1]
[1] State Key Laboratory of Plant Cell and Chromosome Engineering, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing, China 100101
[2] University of Chinese Academy of Sciences, Beijing, China 100049
[3] Division of Biological Sciences, University of Missouri, Columbia, Missouri 65211-7400

Haspin-mediated histone H3-threonine 3 phosphorylation (H3T3ph) play a key role in chromosome segregation in metazoans. However, its significance in regulating the cell cycle remains relatively unexplored in plants. Here, we prepared specific H3T3ph antibody and investigated its distribution pattern through cytogenetic observation in maize mitosis and meiosis. H3T3ph first appeared in prophase, and proceeded to spread along the entire sister chromatids in late prophase and drastically reduced during anaphase in mitosis. Staining was seen between the sister chromatids on spread chromosomes, suggesting a role in sister chromatid cohesion. In maize meiocytes, H3T3 phosphorylation occurred at the late diakinesis stage and extended to the entire chromosome in metaphase I, but was exclusively limited to the centromere at metaphase II. Staining disappeared during meiosis II in the sgo1 mutant because of the loss of centromeric cohesion protector. In the meiosis-specific cohesion mutant afd1, there was also no H3T3ph labeling in the centromeric regions at metaphase II; however, it has weak signals at metaphase I. All in all, the availability of these maize mutants indicate that histone H3T3 phosphorylation is correlated with sister chromatid cohesion. Further we used a YFP-CENH3 transgenic maize line to investigate functional centromere composition in maize. At prometaphase, the centromere contained a mixed distribution of H3T3ph and CENH3 signals. However, CENH3 nucleosomes moved toward the outside of the centromere, and H3 nucleosomes gathered in the inner centromere at metaphase. We hypothesize that the dynamic position changes of nucleosomes within the centromere from prometaphase to metaphase may be related to kinetochore formation and is required to keep the tension between sister chromatids. In addition, we identify a Haspin kinase in maize (ZmHaspin). The purified ZmHaspin has a role in phosphorylating histone H3-threonine 3 in vitro. Further detailed analysis of the Haspin RNAi transgenic plants will provide new insights to the function of H3T3ph in sister chromatid cohesion.

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P261

Taking a look at plant DNA replication: recent insights, new questions, and data sharing through OMERO.bio.fsu.edu

(submitted by Savannah Savadel <sds14d@my.fsu.edu>)
Full Author List: Savadel, Savannah D[1]; Bass, Hank W[1]
[1] Department of Biological Science; Florida State University; Tallahassee, FL, USA 32306-4295

Advances in replicative DNA labeling technology have allowed new ways to study temporal and spatial aspects of DNA replication in living plants. DNA replication timing is coupled to genomic structure and function. Bass *et al.* (*Plant Molec Biol*, 2015) examined the spatiotemporal pattern of DNA replication from developing maize root tips as part of a collaborative study with scientists at NCSU. Three distinct replication patterns were found at Early-S, Middle-S, and Late-S phase. These observations and quantitative measurements led to a two-compartment euchromatic DNA replication model, in which early-S and middle-S are distinguishable in space, time, and DNA condensation. Here we further relate these patterns to those from mammalian DNA replication studies in an effort to identify conserved spatiotemporal counterparts. We also explore the possible significance of the punctate rDNA synthesis inside the nucleolus and speculate about the source and meaning of these signals. Finally, we present a web resource for viewing these 3D data, OMERO.bio.fsu.edu. This platform uses open-source software and data format standards for the storage and manipulation of biological microscopy data. Given the important role of cytogenetics in maize, we seek to use OMERO to overcome chronic limitations in sharing, viewing, and analyzing 3D image data. Public access to the folder “3D Data DNA Rep, ZmRootNuclei” at http://omero.bio.fsu.edu/webclient/?show=project-160 is available through login “Public” password “omero”, with opportunities to house other or published datasets (contact HWB).

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P262

The maize KNL1-Mis12-Ndc80 network for chromosome orientation and segregation during meiosis

(submitted by Handong Su <shdong@genetics.ac.cn>)
Full Author List: Su, Handong[1 2]; Liu, Yalin[1 2]; Liu, Yang[1 2]; Yuan, Jing[1]; Birchler, James A.[3]; Han, Fangpu[1]
[1] State Key Laboratory of Plant Cell and Chromosome Engineering, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing, 100101, China
[2] University of the Chinese Academy of Sciences, Beijing 100049, China
[3] Division of Biological Sciences, University of Missouri-Columbia, Columbia, MO, 65211-7400, USA

The ten-subunit KNL1-Mis12-NDC80 (KMN) network is required for faithful chromosome segregation and genomic stability of all organisms and is an essential component of the kinetochore-microtubule attachment (KT-MT) interface. In maize, the Mis12–Ndc80 Bridge fused the sister kinetochores to initiate the reductional division in meiosis I. However, the detecting homolog of core protein KNL1 in plants was fraught with difficulty, as the pretty complicated evolutionary history.
We identified a KNL1 candidate in maize, which has a conserved RVSF domain in N-terminal and a coiled coil domain in the C-terminal. We identified the MELT-like repeats (MXXX, (x = 2 out of 3 amino acids are D or E) and believe their phosphorylation may be required for spindle assembly checkpoint (SAC) recruitments as reported in human and yeast. Immunoassaying of ZmKNL1 reveals that the signals localized to the kinetochore with constant signal strength during the entail cell cycle as the signals of Mis12 and NDC80. Further immunostainings on maize meiotic mutants afd1 and sgo1 indicate that recruitment of KMN network is not associated with the cohesin and sgo1 proteins in maize. We observed sterile phenotype on ZmKNL1-Mu mutants, with chromosome alignment and segregation errors on meiocytes. This work will shed light on the mechanism of KMN network in maize for chromosome segregation and orientation during cell division.

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P263

The transcriptomic analysis of meiosis initiation in maize male meiocytes

(submitted by Shu-Yun Chen <ubs717@gmail.com>)
Full Author List: Chen, Shu-Yun[1]; Wang, Chi-Ting[1]; Tseng, Ching-Chih[1]; Lin, Wen-Dar[1]; Chen, Pao-Yang[1]; Wang, Chung-Ju Rachel[1]
[1] Institute of Plant and Microbial Biology, Academia Sinica, Taipei, Taiwan

Mitosis and meiosis are essential cell divisions in all eukaryotes. During plant sexual reproduction, germ cells are differentiated late from somatic cells within anthers and ovules, and their cell cycles are then switched from mitotic to meiotic program to produce haploid gametes. With the abilities of defining stages during meiosis initiation and isolating germ cells from maize anthers, we performed mRNA sequencing to investigate the transcriptional dynamics during meiosis initiation, including isolated male meiocyte cells at G1, S, and G2 stages at initial steps of meiosis, shoots and tassel primordium that represent somatic mitosis phase. The RNA-seq data was mapped against maize genome (Zea_may.AGPv3.22) using bowtie2, model and blat. Although more than half of annotated genes showed RPKM higher than 0.1 in meiocytes, 31% of genes are lowly expressed within stages individually. 2,482 genes (7.6%) and 1,397 genes (4.3%) are specifically up- and down-regulated in germ cells during the transition, compared to somatic samples. The up-regulated genes are enriched in the function of transcription initiation from RNA polymerase II promoter, and the down regulated genes are enriched in the function of stress response. In the pairwise comparison for isolated meiocytes between successive stages, significant numbers of genes (~2,500 genes) were differentiated regulated, suggesting the meiosis initiation exhibited a highly complex and dynamic transcriptome. We also identified numerous candidate genes likely to involve in meiosis initiation of maize anthers to be further examined. Our finding provides a roadmap to decipher the molecular mechanism involved in meiosis initiation.

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P264

Variation in meiotic recombination patterns between male and female using high-resolution crossover mapping

(submitted by Penny Kianian <kiani002@umn.edu>)
Full Author List: Kianian, Penny M.A.[1]; Wang, Minghui[2 3]; Simons, Kristen[4]; Ghavami, Farhad[5]; He, Yan[2 6]; Dukowic-Schulze, Stefanie[1]; Sundararajan, Anitha[7]; Qi, Sun[3]; Pillardy, Jaroslow[3]; Mudge, Joann[7]; Kianian, Shahryar[8]; Chen, Changbin[1]; Pawlowski, Wojciech P.[2]
[1] Department of Horticultural Science, University of Minnesota, St. Paul, MN, USA 55108
[2] Section of Plant Biology, School of Integrative Plant Science, Cornell University, Ithaca, NY, USA 14853
[3] Computational Biology Service Unit, Cornell University, Ithaca, NY, USA 14853
[4] Department of Plant Sciences, North Dakota State University, Fargo, ND, USA 58102
[5] Eurofins-BioDiagnostics, River Falls, WI, USA 54022
[6] National Maize Improvement Center, China Agricultural University, Beijing, China
[7] National Center for Genome Research, Santa Fe, NM, USA 87505
[8] Cereal Disease Laboratory, USDA-ARS, St. Paul, MN, USA 55108

Meiotic recombination is the major source of genetic variation in evolution, and is necessary for improvement of plant and animal breeds. Despite the importance of the resulting crossovers (COs), they are not uniformly distributed across the genome, a phenomenon which is not completely understood. One of the least elucidated aspects of meiotic recombination are variations between CO rates and patterns between male and female meiosis. To investigate these differences, we conducted high-resolution mapping of CO locations using whole-genome sequencing in the maize B73 x Mo17 hybrid. We found the overall CO rates were similar in the two sexes. However, the positions of recombination hotspots do not overlap between the sexes and significant differences exist between male and female CO rates in several Mb-long genome regions. Furthermore, the placement of the CO in relationship to gene transcription start sites varied between the sexes. Epigenetics factors, such as DNA methylation, nucleosome occupancy, and histone modifications were also found to have effects on CO formation in both sexes. Using our high-resolution CO map, it was also possible to explore differences between class I (interference sensitive) and class II (interference insensitive) COs, which so far has only been possible using low-resolution cytogenetics methods. These results will aid in deciphering the mechanisms regulating CO rates and locations in plants.

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P265

CRISPR-Cas advanced breeding to produce next generation waxy corn products

(submitted by Robert Meeley <bob.meeley@pioneer.com>)
Full Author List: Basu, Suthirta[1]; Betts, Scott[1]; Bryant, Morrie[1]; Fedorova, Maria[1]; Gadlage, Mark[1]; Gao, Huirong[1]; Meeley, Robert[1]
[1] DuPont Pioneer, Johnston, Iowa, USA 50131-1004

CRISPR-Cas genome editing technology is being rapidly deployed as an Advanced Breeding Technique to introduce new commercial Waxy hybrids to the marketplace. Whole gene deletions of the *Wx1* granule-bound starch synthase gene have been precisely generated in a broad mix of elite NSS and SSS inbreds. Hybrids generated from this panel are headed for their first comprehensive set of yield tests in Summer, 2017. An update on the strategy used to generate and evaluate these lines will be presented.

P266

Genome quilt for maize teosinte branched 1: A unique opportunity for public outreach

(submitted by Addie Thompson <thomp464@purdue.edu>)
Full Author List: Thompson, Deborah K[1]; Thompson, Addie M[2]
[1] Sew Many Stitches; www.sewmanystitches.co; O'Fallon, IL, USA 62269
[2] Purdue University; West Lafayette, IN, USA 47907

Genome Quilts (genomequilts.com) have been used as a way for people to share their personal history and passions. With these quilts, people code a length of DNA sequence in the patterning of the pieces of fabric. In a team of a quilter (Debbie Thompson, Sew Many Stitches, www.sewmanystitches.co) and a maize researcher (Addie Thompson, Purdue University), we chose the maize gene teosinte branched 1, the major gene involved in the domestication of maize from its wild ancestor, teosinte. A sequence change in this gene changed the shape of teosinte from a bushy plant with many branches into the single stalk of corn we grow today. The goal was to create a piece of artwork that could be used as an outreach tool, sparking interest in textiles and agricultural genetics. Beyond coding a sequence from maize in the pattern, the quilt is also made entirely from corn products. The thread, fabric, and batting are all experimental products from companies around the world interested in donating or selling corn products for this project. This quilt is currently being scheduled for display at public institutions and private companies interested in promoting maize, genetics, agriculture, and the arts.

P267

Laboratory techniques in plant molecular biology taught with UniformMu insertion alleles of maize

(submitted by Christine Chase <cdchase@ufl.edu>)
Full Author List: Cathey, Rebecca B[1]; Coskan, Sevgi[1]; Maxwell, Duncan J[1]; Elliott, Kiona R[1]; Joseph, Ryan[1]; Lim, Adrienne C[1]; McManus, Thomas F[1]; Rodgers, John M[1]; Shimansky, Andrew J[1]; Vereen, Christina D[1]; Xie, Yucong[1]; Hunter, Charles T[2]; Suzuki, Masaharu[1]; Koch, Karen E[1]; McCarty, Donald R[1]; Chamusco, Karen C[1]; Chase, Christine D[1]
[1] University of Florida Horticultural Sciences Department, Gainesville, FL USA 32611-0690
[2] USDA-ARS Center for Medical, Agricultural and Veterinary Entomology / Chemistry Research Unit, Gainesville, FL 32608

An undergraduate course - Laboratory Techniques in Plant Molecular Biology - was organized around our research application of UniformMu insertion alleles to investigate mitochondrial functions in plant reproduction. The course objectives were to develop students' laboratory, record keeping, bioinformatics, and critical thinking skills, enabling them to work comfortably and safely in a molecular biology laboratory environment and use current web-based tools for the analysis of nucleic acid and protein sequences. S-type cytoplasmic male sterility (CMS-S) in maize is characterized by a mitochondria-encoded pollen collapse phenotype that can be reversed by nuclear genetic mutations. These include numerous *restorer-of-fertility-lethal (rfl)* alleles that additionally condition homozygous-lethal seed phenotypes. Students investigated UniformMu insertion alleles of 10 nuclear genes having predicted mitochondrial functions as candidate *rfl* alleles. Expression patterns of non-mutant alleles were investigated in pollen and seed development through end-point, reverse-transcriptase PCR (RT-PCR). Mitochondrial targeting leaders were predicted for the non-mutant protein products through amino acid sequence comparisons with orthologous proteins. PCR assays were developed for the insertion alleles, and plants heterozygous for insertion alleles were self or sib pollinated to investigate seed phenotypes. Insertion positive plants were also used to pollinate CMS-S plants for analysis of pollen transmission frequencies in normal cytoplasm and pollen fertility restoration in S-cytoplasm.

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P268

NSF research traineeship – P3, Predictive Plant Phenomics

(submitted by Carolyn Lawrence-Dill <triffid@iastate.edu>)
Full Author List: Dickerson, Julie[1]; Heindel, Theodore[1]; Lawrence-Dill, Carolyn J.[1]; Schnable, Patrick S.[1]
[1] Iowa State University

New methods to increase crop productivity are required to meet anticipated demands for food, feed, fiber, and fuel. Using modern sensors and data analysis techniques, it is now feasible to develop methods to predict plant growth and productivity based on information about their genome and environment. However, doing so requires expertise in plant sciences as well as computational sciences and engineering. Through P3, we bring together students with diverse backgrounds, including plant sciences, statistics, and engineering, and provide them with data-enabled science and engineering training. The collaborative spirit required for students to thrive in this unique intellectual environment will be strengthened through the establishment of a community of practice to support collective learning. This traineeship anticipates preparing forty-eight (48) doctoral students, including twenty-eight (28) NRT funded doctoral students, with the understanding and tools to design and construct crops with desired traits that can thrive in a changing environment. Visit the P3 website at https://www.predictivephenomicsinplants.iastate.edu to learn more.

Funding acknowledgement: National Science Foundation (NSF)

P269

A 4bp insertion at ZmPLA1 generates haploid induction in maize

(submitted by Chenxu Liu <liuchenxusdau@126.com>)
Full Author List: Liu, Chenxu[1]; Li, Xiang[2]; Meng, Dexuan[1]; Zhong, Yu[1]; Chen, Chen[1]; Dong, Xin[1]; Li, Liang[1]; Xu, Xiaowei[1]; Chen, Baojian[1]; Li, Wei[1]; Tian, XiaoLong[1]; Chen, Ming[1]; Zhao, Haiming[1]; Song, Weibin[1]; Luo, Haishan[1]; Zhang, Qinghua[2]; Lai, Jinsheng[1]; Jin, Weiwei[1]; Yan, Jianbing[2]; Chen, Shaojiang[1]
[1] China Agricultural University; Beijing, China 100193
[2] Huazhong Agricultural University; Wuhan, China 430070

Doubled haploid (DH) technology based on in vivo haploid induction (HI) is used to accelerate the efficiency of breeding widely on maize and other crops. In vivo haploid induction by Stock6 derived inducers can lead to maternal haploid and has been considered as the most effective method for DH breeding in maize. A major QTL-qhir1 located on chromosome 1 was narrowed down into 243kb region. Based on cell type expression analysis, bioinformatics gene annotation, BAC library screening and CRISPER/Cas9 gene editing, ZmPLA1 encoded phospholipase A (GRMZM2G471240) was verified the underlying gene. In total, 11 SNPs and a 4bp insertion was found in the exons within ZmPLA1. Candidate gene sequence analysis in 300 maize regular inbred lines, 180 teosinte accessions and six inducer lines demonstrated that the 4bp (CGAG) insertion was only present in the inducer lines not in non-inducer lines which may cause a weak or loss-of-function allele of ZmPLA1 leading to the haploid induction phenotype. Three independent CRISPER/Cas9 knockout lines were generated and used to test the HI efficiency by crossing with two commercial hybrids and the average HI rate of different knockout lines ranged between 1.85-3.51% with an average 2%, which is close to the HI rate of Stock6, the initial inducer line. Our results suggest that ZmPLA1 is the underlying gene of qhir1 and the rare 4bp insertion occurred post domestication is the casual allele which could lead to haploid induction.

P270

A new dominant dwarfing mutant of maize exhibiting exquisite sensitivity to the genetic background

(submitted by Amanpreet Kaur <kaur60@purdue.edu>)
Full Author List: Kaur, Amanpreet[1]; Salleres-Neira, Belén[1]; DeLeon, Alyssa[1]; Dilkes, Brian[2]; Johal, Gurmukh Singh[1]
[1] Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN,USA 47907
[2] Department of Biochemistry, Purdue University, West Lafayette, IN,USA 47907

Dwarf plants have had a great impact on world agriculture. The ushering of the green revolution in the 1960s and 70s was due to the development of dwarf varieties of wheat and rice. Short plants perform better because of their ability to resist lodging caused by wind/rain, and this allows them to respond better to agricultural inputs and produce higher yields. Dwarf plants can also help reduce the environmental footprint of agriculture as they need relatively fewer inputs. In maize several dwarf mutants have been reported, including those that are defective in the biosynthesis or signaling of key phytohormones, including auxins, brassinosteroids and gibberellins. However, we keep identifying new ones, especially those that exhibit a dominant or semi-dominant inheritance. Recently, we identified one such mutant in an M1 population of B73 generated by mutagenesis with EMS. Designated D13*, this dwarfing mutant does not seem to match any of the known mutants of maize. In addition, the phenotype of D13* is dramatically impacted by the genetic background, being enhanced in some backgrounds and completely suppressed in others. While its phenotype is quite severe in B73, ranging in size from 1-2 feet, D13* almost reverts to wild-type in the Mo17 background. To genetically dissect this suppression of D13*, we made use of the MAGIC enhancer/suppressor screen by crossing D13* to IBM RILs and phenotyping the resulting testcross progenies. This analysis allowed us to identify a significant QTL that mapped to chromosome 5. We are now trying to clone the gene underlying this QTL, as well as the D13* gene itself, in collaboration with Bailin Li's group at Dupont/Pioneer.

P271

A sorghum NAC gene affects vascular development and biomass properties

(submitted by Jingnu Xia <jxia5@illinois.edu>)
Full Author List: Xia, Jingnu[1]; Zhao, Yunjun[2]; Burks, Payne[1 3]; Pauly, Markus[2 4]; Brown, Patrick[1]
[1] Department of Crop Sciences, University of Illinois at Urbana Champaign, Urbana IL 61801
[2] Department of Plant and Microbial Biology, University of California, Berkeley, Berkeley, CA, 94702
[3] Chromatin Inc, Lubbock TX 79424
[4] Heinrich-Heine University, Duesseldorf, Germany

Sorghum bicolor is not only an important cereal crop but also an emerging bioenergy feedstock. The sorghum *Dry Stalk (D)* locus has long been known to condition the difference between juicy green *(dd)* versus *dry white (D-)* midribs. Here we show that allelic differences at *D* also affect biomass moisture, and fine-map the *D* locus to a 38 Kb region containing four genes, two of which are expressed in midrib tissue. One of the two expressed genes is a NAC transcription factor that carries a premature stop codon in *d* but not in the *D* alleles. The NAC family transcriptional factors contains master regulators of secondary cell wall development, including the vascular-related NAC domain (VND) and NAC secondary wall thickening promoting factor (NST) proteins that regulate the development of xylem vessels and fibers, respectively. The sorghum NAC gene in the *D* interval is orthologous to NAC074 in Arabidopsis, which is repressed by SND1. Here, we present transcriptomic compositional, and anatomic measurements from BC3-derived near-isogenic lines for the *D* locus.

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P272

Accelerating large scale plant breeding and product development

(submitted by Ruth Wagner <ruth.wagner@monsanto.com>)
Full Author List: Wagner, Ruth A[1]; Swarup, Shilpa[1]; Wanjugi, Humphrey[1]; Osborn, Tom[1]
[1] Monsanto Company; St. Louis, MO USA

The identification of high quality, trait-associated genetic markers is reliant on recombination, phenotyping and the identification of polymorphic markers in chromosome regions of interest. At Monsanto, trait-linked marker development and deployment for yield, disease and agronomic traits is accelerated through the use lab automation as well as development and deployment of next generation genotyping and sequencing technologies, with the goal to identify genetic markers for use in high throughput genotyping assays to enable marker-assisted breeding. We highlight examples of deploying new technologies, leveraging public research, and leveraging multiple genotyping technology platforms to enable integration of large amounts of genomic data to accelerate commercial breeding.

P273

Advancing understanding of heat stress response mechanisms by integrated molecular, biochemical, and whole-plant analysis

(submitted by James McNellie <mcnellie@iastate.edu>)
Full Author List: McNellie, James P.[1]; Li, Xianran[1]; Chen, Junping[2]; Yu, Jianming[1]
[1] Department of Agronomy, Iowa State University, Ames, Iowa
[2] USDA-ARS, Plant Stress & Germplasm Development Unit, Lubbock, Texas

Periods of high temperature stress reduce maize yields, and both the frequency and duration of deleteriously high temperatures are expected to increase. A better understanding of the genetic architecture and underlying molecular mechanisms of maize heat stress response will help guide future breeding efforts. Our objective is to establish a clearer understanding of heat stress response by integrating whole-genome transcriptome profiling (i.e., co-expression network and differential network analysis), extensive physiological characterization of diverse maize inbreds, with robust genetic mapping of targeted populations. A set of 24 diverse inbred lines will be grown in a controlled environment under heat stress and non-stress conditions. RNA sequencing will be performed so that genes critical to the heat stress response can be identified via Differential Network analysis. The photosynthetic and leaf-level net carbon assimilation will also be phenotyped. Two mapping population are being developed. The first one, B76/B106, is a doubled haploid (DH) population with 234 individuals. The second one, B76/NC350, is a recombinant inbred line population with 126 individuals. As a remedy to the relatively small size of the second population (due to the adaptation issue from NC350), we have also developed a third population, NC350/B106, with 150 individuals. In addition, we analyzed two mapping populations that were previously phenotyped for heat stress response in field conditions (CML103×B73 and NC350×B73). By integrating physiology and transcriptome analysis with field based heat stress trials, we hope to elucidate mechanistic connections between genes, pathways, physiological development and whole-plant stress response.

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P274

An optimized and cost-efficient maize genotyping array for routine use in maize breeding

(submitted by Martin Ganal <ganal@traitgenetics.de>)
Full Author List: Ganal, Martin[1]; Plieske, Joerg[1]; Polley, Andreas[1]; Ventelon-Debut, Marjolaine[2]; Charcosset, Alain[3]; Falque, Matthieu[3]
[1] TraitGenetics GmbH, Gatersleben, Germany
[2] Euralis Semences, Mondonville, France
[3] INRA, Gif sur Yvette, France

For routine genotyping within the maize breeding process it is necessary to have a cost-efficient genotyping system available. In order to provide the maize breeding community an optimized genotyping array, we have generated a new Illumina Infinium array based on the MaizeLD array containing 3,047 markers for EDV identification (Rouselle et al., 2015) by adding 11,615 SNP markers from the Maize SNP50 Illumina array. Selection of these additional markers was based on the distribution of the markers mainly based on recombination (genetic map position and the detection of specific haplotype blocks), marker quality and call frequency. With low costs per sample (between 30 and 40 $, including array, DNA extraction, genotyping and generation of standardized allele tables directly for data analysis) for large sample numbers (>480), this array is now widely used in maize genotyping for purposes such as genetic mapping, diversity analysis of maize lines, and genomic selection.

P275

Analysis of extreme phenotype copy number variation (XP-CNV) reveals an association of the rust resistance locus *Rp1* with resistance to Goss's Bacterial Wilt and Leaf Blight

(submitted by Ying Hu <huying@ksu.edu>)
Full Author List: Hu, Ying[1]; Ren, Jie[1]; Peng, Zhao[2]; Danilova, Tatiana[1]; Hulbert, Scot H.[3]; White, Frank F.[2]; Liu, Sanzhen[1]
[1] Department of Plant Pathology, Kansas State University, Manhattan, KS. 66506. U.S.A.
[2] Department of Plant Pathology, University of Florida, Gainesville, FL. 32611. U.S.A.
[3] Department of Crop and Soil Sciences, Washington State University, Pullman, WA, 99164. U.S.A.

Goss's bacterial wilt and leaf blight of maize, which is commonly referred to as Goss's wilt (GW), was first identified in Nebraska in 1969 and reemerged in major maize-producing areas of North America and Canada in recent years. Clavibacter michiganensis subsp. nebraskensis (Cmn), the causal agent of GW is a gram positive bacterium and causes systemic wilting on young plants and leaf blighting at any stage of growth. The genetic basis of plant resistance to GW remains unknown. A simple and reliable method for high-throughput quantification of disease symptoms on maize seedlings was developed, enabling mapping of the genetic components conferring GW resistance, and genome-wide association (GWAS) analysis revealed GW resistance associated loci. Whole-genome-shotgun (WGS) sequencing was also performed on two resistant plant pools (R) and two susceptible pools (S) sampled from the highly resistant and highly susceptible maize lines. WGS data were used to examine allele frequencies of polymorphic sites between the R and S groups (extreme phenotype-GWAS or XP-GWAS) as well as to determine copy number variation (CNV) between pooled samples (XP-CNV). An association of CNV of three paralogous genes at the *Rp1* locus with disease resistance was identified. Quantitative real-time PCR and analysis using an independent set of WGS data of ~200 maize lines confirmed the association. In addition, multiple *Rp1* alleles that were introgressed into the inbred susceptible line H95 showed significantly enhanced resistance to GW in comparison to H95. The results will facilitate the characterization of GW resistance genes and breeding GW resistant maize varieties.

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P276

Analysis of gene expression changes in response to cold stress in maize seedlings

(submitted by Jia Tan <jtan03@hamline.edu>)
Full Author List: Tan, Jia W[1]; Larsen, Siri C[1]
[1] Hamline University; 1536 Hewitt Ave, St Paul, MN 55104

Recent climate change has caused overall colder seasons across the world. Low temperature has been documented to have multiple negative effects on plants, including delayed growth, tissue damage and if severe, death. Maize, despite being a cold susceptible species, shows some degree of cold tolerance. Gene expression analysis is a useful tool to identify genes in maize varieties that may be responsible for acclimation to the cold. Several genes involved in controlling response to cold stress were identified using gene expression analysis, however, many other genes are likely involved in this process and finding them could help in breeding hardier varieties more resistant to cold stress. In this study, time course global gene expression studies were utilized to discover candidate genes involved in cold tolerance response. Quantitative real-time polymerase chain reaction (qRT-PCR), was then used to characterize and compare gene expression in three maize varieties with different degrees of tolerance to cold. qRT-PCR results confirmed that most of the selected candidate genes are likely to be involved in cold tolerance response. In addition, genes were analyzed and grouped based on their behavior during cold stress in cold tolerant and cold sensitive maize varieties. This analysis suggested that some of the selected genes, based on their conserved behavior, may be part of a large group of genes that are responsible for cold tolerance in some maize varieties. This study demonstrated that a large proportion of maize genes are activated or down-regulated in response to cold, and that regulation of gene expression varies in cold tolerant and cold susceptible varieties. Although further studies are necessary to identify specific genes conferring cold tolerance in maize, results provided novel information about candidate genes that control cold response in maize seedlings.

Funding acknowledgement: National Science Foundation (NSF)

P277

Analysis of the genetics architecture of fruitcase shapes in a natural teosinte population

(submitted by Kyle Krueger <kwkrueger@wisc.edu>)
Full Author List: Krueger, Kyle W[1]; Panzea Group, The[1 2 3 4 5]; Doebley, John F[1]
[1] University of Wisconsin - Madison; 425 Henry Mall; Madison, WI, USA 53706
[2] USDA-ARS
[3] North Carolina State University; Raleigh, NC, USA 27695
[4] University of California; Davis, CA, USA 95616
[5] Cornell University; Ithaca, NY, USA 14853

Maize (*Zea mays* ssp. *mays*) is one of the most common food crops existing today that has been domesticated throughout history to tailor to human needs. This “tailoring” is estimated to have begun around 9000 years ago starting in the Balsas river valley of southern Mexico. Since then, modern researchers have been able to dissect the underlying genetic architecture of domestication traits and improved their understanding of the domestication process. Although scientists have been able to discover the origins of maize, there has been very little research done on identifying the available genetic variations in teosinte that could be responsible for domestication. By utilizing a quantitative imaging and analysis software coupled with a quantitative genetic approach, we have been able to identify significant genetic architecture variations in teosinte fruitcase structures such as: length, width, length-width-ratio, triangularity, and circularity. Some of the genes discovered that dictate fruitcase shape and size could have been targeted for domestication by our ancestors. Selection for teosinte fruitcases with higher triangularity and shorter length provided a more densely packed ear, yielding more food within a single ear. Based on our results from a modern teosinte population, we can translate that to the founding teosinte population to understand how fruitcase shapes evolved during the domestication process.

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P278

Applying hyperspectral imaging to studying temperature stress responses in maize

(submitted by Tara Enders <tenders@umn.edu>)
Full Author List: Enders, Tara A[1]; Springer, Nathan M[1]
[1] University of Minnesota; 140 Gortner Laboratory, 1479 Gortner Ave; Saint Paul, MN, USA 55108

Average yields of maize could drop substantially within the next century due to global climate change. Understanding how maize varieties respond to temperature extremes will be instrumental in developing varieties that can withstand future temperature stresses while still producing high yield. Hyperspectral imaging of maize in response to abiotic stress is an unexplored concept, and is likely to provide useful data to researchers interested in both abiotic stress and hyperspectral imaging. Hyperspectral imaging tools are being developed to study phenotypes of temperature-stressed maize seedlings. Current efforts include minimizing environmental and plant morphological effects on obtained data, as well as developing methods for data analysis in MATLAB and R. We will document the variation of hyperspectral traits in maize seedlings in response to both high and low temperature stresses in multiple maize genotypes. Documenting spectra across genotypes and growth conditions will uncover the dynamics of maize spectra in response to changing temperatures and allow for the discovery of genomic loci that could provide improved tolerance.

P279

Canalization in the phosphate-starvation response of a Mexican maize landrace native to acidic volcanic soils

(submitted by Elsa Ibarra Reyes <elsa.ireyes@gmail.com>)
Full Author List: Ibarra Reyes, Elsa[1]; Gonzalez Segovia, Erick G.[1]; Chávez Montes, Ricardo A.[1]; Aguilar Ranger, María Rocio[2]; Torres Rodríguez, J. Vladimir[1]; Salazar-Vidal, M. Nancy[1]; Rellan Alvarez, Ruben[1]; Simpson Williamson, June K[2]; Sawers, Ruairidh J. H.[1]; Herrera-Estrella, Luis[1]
[1] Laboratorio Nacional de Genómica para la Biodiversidad (LANGEBIO), Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional (CINVESTAV-IPN), Irapuato, Guanajuato, Mexico, C.P. 36821,
[2] Centro de Investigación y Estudios Avanzados (CINVESTAV-IPN), Departamento de Ingeniería Genética de Plantas, Irapuato, Guanajuato, Mexico, C.P. 36821,

Locally adapted landraces and crop wild-relatives, the products of past selection in diverse environments, represent a useful system to better understand stress responses and a valuable source of genetic variation. In maize, domestication in the Balsas valley of southwestern Mexico was followed by rapid dispersal beyond the ancestral niche, including spread to the Mexican central highlands, a region with an elevation of >2000 m above sea level, cooler temperatures, drier climate and acidic volcanic soils, characterised by low phosphorus availability. To investigate molecular patterns of adaptation to low phosphorus availability in Mexican highland maize, we have performed a comparative analysis of an Allele Specific Expression (ASE) transcriptome dataset generated from the F1 hybrid of B73 x Palomero Toluqueño (PT) and a B73 low phosphate stress transcriptome. We have identified a small panel of genes that under normal conditions are expressed differentially between B73 and PT alleles in a manner that mirrors the response of B73 to low phosphate availability. We consider these genes to display regulatory canalization, with a potential role in local adaptation.

Funding acknowledgement: CONACyT

P280

Canalization in the regulation of stress-responsive transcripts identifies candidates of potential adaptive importance in the Mexican highland maize landrace Palomero Toluqueño

(submitted by Maria Rocio Aguilar Rangel <arangel.mro@gmail.com>)
Full Author List: Aguilar Rangel, María R[1 2]; Chávez Montes, Ricardo A[1]; González Segovia, Eric G[1]; Ross-Ibarra, Jeffrey[3]; Simpson, June[2]; Sawers, Ruairidh J. H.[1]
[1] Laboratorio Nacional de Genómica para la Biodiversidad (LANGEBIO), Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional (CINVESTAV-IPN), Irapuato, Guanajuato, Mexico. C.P. 36821
[2] Centro de Investigación y Estudios Avanzados del Instituto Politécnico Nacional (CINVESTAV-IPN), Departamento de Ingeniería Genética de Plantas, Irapuato, Guanajuato, Mexico. C.P. 36821
[3] The Center for Population Biology and the Genome Center, University of California, Davis, California 95616 , USA.

Since domestication in southwest Mexico, maize has dispersed and adapted to a great variety of environments facing dramatically different environmental including the highlands of Mexico where adaptation to drier environments, lower temperatures, higher solar radiation and reduced atmospheric pressure has produced a unique group of landraces that represent a valuable genetic resource.
Change to gene cis regulatory elements (CREs) is considered a key mechanism by which novel functional alleles are generated to confer greater stress tolerance in locally adapted crop varieties. Here, we perform a comparative analysis of Allele Specific Expression (ASE) between the highland landrace Palomero Toluqueño (PT), which is well adapted to the highlands environment, and the US reference line B73 to identify cis regulatory variation on the basis of an F1 transcriptome from greenhouse seedlings. Such genes were compared with reported stress responsive genes in B73 and we identified a set of genes whose expression levels in PT allele corresponds with induced or repressed expression levels in B73 seedlings under heat, salt, cold and UV stress. We will explore these genes in broader Mexican landrace diversity in the context of identifying candidates for enhancing cereal adaptation to marginal lands.

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P281

Characterization of Landrace Piura-derived maize silk resistance to corn earworm herbivory using a scaled-up quantitative bioassay

(submitted by Miriam Lopez <miriam.lopez@ars.usda.gov>)
Full Author List: Lopez, Miriam[1 2]; Posekany, Tes[2 3]; Paque, Tina[1 4]; Yandeau-Nelson, Marna D.[3 5]; Adams, Nancy J.[6]; Abel, Craig[1 4]; Lauter, Nick[1 2 3]
[1] Corn Insects and Crop Genetics Research Unit, USDA-ARS, Ames, Iowa, USA, 50011
[2] Department of Plant Pathology and Microbiology, Iowa State University, Ames, Iowa, USA, 50011
[3] Genetics and Genomics Graduate Program, Iowa State University, Ames, Iowa, USA, 50011
[4] Department of Entomology, Iowa State University, Ames, Iowa, USA, 50011
[5] Department of Genetics, Development and Cell Biology, Iowa State University, Ames, Iowa, USA, 50011
[6] Southern Entomology Development Unit, Monsanto Corporation, Union City, Tennessee, USA, 38261

Corn earworm (CEW), *Helicoverpa zea* (Boddie), (Lepidoptera: Noctuidae), is a major insect pest of corn, whereon its larvae cause substantial yield and quality losses by feeding on silks, kernels, and cob tissue. The long-term goal of this work is to elucidate the genetic and biochemical basis of a previously reported non-maysin source of CEW resistance first observed in silk tissue of Piura 208, a Peruvian Landrace of maize (PI 503849). To enable execution of future genomic and metabolomic experiments, we developed a quantitative CEW bioassay and tested it on four small populations that contrast alleles from Piura 208 with those from GT119, a susceptible maize inbred that also lacks maysin production. In replicated analyses of two populations of $F_{1:2}$ families, corn genotype accounts for 84% ($p<0.0001$) and 68% ($p<0.0001$) of the variance in CEW larval weights, demonstrating both the success of the quantitative bioassay and the strength of the Piura 208 resistance mechanism. Corresponding analysis of two populations of $BC_{1:2}$ families revealed no statistically significant effects of corn genotype, suggesting multigenic inheritance of resistance and/or a failure to capture key resistant alleles in backcrossing, two possibilities whose contributions cannot be quantified in the study. Technical factors in bioassay performance were assessed, primarily by analyzing 1641 CEW larvae raised on control diet (meridic with no corn silks added). Minor, but statistically significant contributions to CEW weight gain variance were revealed for factors in the preparation, incubation and evaluation phases of the bioassay, demonstrating the importance of randomization, stratification, replication, and variable-tracking across the many steps of the quantitative CEW bioassay. Together, these findings indicate that the novel CEW resistance originating in Piura 208 is experimentally tractable using this bioassay and the alleles already captured in the $F_{1:2}$ families. Plans for breeding additional analysis populations are discussed.

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P282

Characterization of maize mutants for drought tolerance and high yield

(submitted by Xia Zhang <zhangxia@caas.cn>)
Full Author List: Zhang, Xia[1]; Ding, Wanhong[2]; Tang, Yong[2]; Lu, Xiaoduo[1]; Feng, Huaizhang[2]; Zhang, Chunyi[1]; Zhao, Jun[1]
[1] Faculty of Maize Functional Genomics, Biotechnology Research Institute, National Key Facility for Crop Gene Resources and Genetic Improvement, Chinese Academy of Agricultural Sciences, Beijing 100081, China
[2] Experimental Station, Xinjiang Academy of Agricultural Sciences, Urumqi 830012, China

Maize (*Zea mays L.*) is an important cereal crop with the largest contribution to the global cereal production. Water deficit especially drought occurring at flowering and early seed development is one of the major factors constraining or destabilizing maize grain production. Substantial yield loss under drought is largely attributed to the potential lack of synchrony between silk emergence and pollen shed. In order to identify high yielding and drought tolerant maize varieties, we made an effort to screen Ethyl Methane Sulphonate (EMS) induced maize mutant population. Drought was imposed at flowering stage by withdrawal of irrigation in field trials. Mutant lines displaying short anthesis-silking interval (ASI) (ASI ≤ 2) and alleviated leaf senescence (greater stay-green) were selected and crossed to generate hybrids. Grain yield of hybrids were evaluated in six replications of two-row plots under drought stressed and well-watered experiments. In field trials of two seasons, we identified several desirable mutants which consistently exhibited an average of 20% increase in grain yield and improved drought performance. The identified drought-tolerant mutants also showed better performance for physiological parameters including photosynthesis, relative water content, cell membrane stability, and chlorophyll concentration upon drought stress in pot experiments. We generated F2 progeny by crossing the drought-tolerant mutant with its wild type followed by selfing of F1 individuals. The F2 individuals that segregate for contrasting morphological traits related to ASI and leaf senescence will allow for further characterizing causative genes or alleles by NGS-assisted MutMap approach.

P283

Characterization of teosinte mexicana introgression in the mexican highland maize landrace Palomero Toluqueño

(submitted by Eric Gonzalez <eric.gonzalez@cinvestav.mx>)
Full Author List: Gonzalez, Eric[1]; Salazar, Nancy[1]; Aguilar, Rocio[1]; Cintora, Carolina[1]; Ramirez, Rosario[1]; Sawers, Ruairdh[1]
[1] Langebio-Cinvestav; Irapuato, Guanajuato, Mexico, 36821;

The aim of this work is to study maize adaptation to the central highlands of Mexico. It is thought that introgression events from its wild relative, the teosinte mexicana, a teosinte endemic from the Mexico highlands, is in part responsible of the adaptation of maize to this environment. To study this phenomenon, I am using the maize highland landrace Palomero Toluqueño (PT), which is considered one of the first highland landraces and posses the highest levels of mexicana introgression. Using genomic information available and generated de novo, I have determined specific regions of introgression in the PT genome. Now I am characterizing these regions, using a linkage map derived from the cross of PT and the inbreed line B73, I have found that big introgressed regions are related to low recombination regions –low recombination regions are thought to be important in the process of adaptation in the presence of gene flow– now I am going to compare these low recombination regions with those ones that I will found in the cross of PT and the mexican lowland landrace Reventador. This cross is representative of a lowland and highland landrace that, under a traditional framing process in Mexico, has been adapted during thousands of years to contrasting environments in the presence of gene flow. This cross will also be useful to map QTLs related with highland phenotypes and I will evaluate if introgressed and low recombination regions are related with these QTLs. To identify regions for highland adaptation and characterize them, it is important to develop strategies in order to use them in crop improvement and allow maize elite lines to grow in different and contrasting environments to face climate change by taking advantage of maize diversity which in turn we must preserve and continue studying.

Funding acknowledgement: CONACYT

P284

Characterizing root-root interactions in U.S. maize: The effects of breeding for high-density crops on Root System Architecture (RSA)

(submitted by Kari Miller <kmiller@danforthcenter.org>)
Full Author List: Miller, Kari D[1]; Ellis, Nathanael A[1]; Edwards, Jode[2]; Topp, Christopher N[1]
[1] Donald Danforth Plant Science Center, St. Louis, MO 63132
[2] USDA-ARS Corn Insects and Crop Genetics Research Unit, Ames, IA 50011

Maize production methods have changed dramatically over the last century resulting in a 600% increase in production, despite a 20% decrease in acreage. A primary driver of this phenomenon is selective breeding for maize crops adapted to growth at high planting densities. While studies have been conducted on above-ground adaptations to high density, little is known about below ground phenotypes, primarily due to the difficult nature of accessing and viewing root systems. However, advances in computational analysis software has allowed for an increase in high-throughput quantification of root system architecture (RSA), both in two and three dimensions. To observe the principal differences in RSA between high- and low-density maize plants, two populations of interest were explored: Iowa Stiff Stalk Synthetic (BSSS) and Iowa Corn Borer Synthetic (BSCB), along with a hybrid of the two. Among these populations, "Cycle 0" represented the genotype before recurrent selection, while "Cycle 17" is after 60 years of selection for high yield at high planting densities. To incorporate environmental effects, an in-lab germination paper experiment and a field experiment were conducted, both of which explored the effects on RSA of interplanting and high/low planting densities. Root traits were quantified using analysis software GiA-Roots as well as Digital Imaging of Root Traits (DIRT). This experiment and analysis indicated that (1) root systems become smaller when grown at high planting densities, both in total network length and depth, and (2) density-adapted maize (i.e. cycle 17) shows a higher level of plasticity then non-adapted, displaying a greater difference in RSA phenotype between low and high density environments. This study expanded experimental technique and analysis methods of root systems, thus paving the way for future studies of root-root interactions, particularly at high densities.

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P285

Characterizing the profile of allele-by-environment interactions using B73-Mo17 introgression lines

(submitted by Sara Tirado <tirad014@umn.edu>)
Full Author List: Tirado, Sara B.[1]; Li, Zhi[1]; Miller, Nathan D.[3]; Spalding, Edgar[3]; de Leon, Natalia[4]; Kaeppler, Shawn M.[4]; Schnable, Patrick S.[5]; Hirsch, Candice N.[1]; Springer, Nathan M.[2]
[1] Department of Agronomy and Plant Genetics, University of Minnesota, Saint Paul, MN 55108
[2] Department of Plant and Microbial Biology, University of Minnesota, Saint Paul, MN 55108
[3] Department of Botany, University of Wisconsin, Madison, WI 53706
[4] Department of Agronomy, University of Wisconsin, Madison, WI 53706
[5] Department of Agronomy, Iowa State University, Ames, IA 50011

Genomic introgressions containing quantitative trait loci (QTL) can have significant impacts on the phenotypic outcome for an organism. The magnitude of phenotypic effects can vary substantially in different environments due to allele-by-environment interactions. A population of B73xMo17 near-isogenic lines (NILs) including 186 B73-like genotypes with Mo17 introgressions and 70 Mo17-like genotypes with B73 introgressions was developed. This population was evaluated for 12 plant morphology and ear morphology traits. A subset of 15 NILs with altered phenotypes relative to the recurrent parent were selected for growth and evaluation in 16 environments along with the parental lines B73 and Mo17. These environments included five geographical locations with multiple planting dates and multiple planting densities. The phenotypic impact of the introgressions was evaluated on up to 25 traits in each environment to assess allele-by-environment interactions. Understanding the profile of allele-by-environment interaction will be useful in considerations of how transgenes for quantitative traits or QTL introgressions might be expected to impact traits in diverse environments.

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P286

Combining ability and heterotic patterns of early-maturing provitamin A inbreds under contrasting environments

(submitted by Baffour Badu-Apraku <b.badu-apraku@cgiar.org>)
Full Author List: Badu-Apraku, Baffour[1]; Talabi, Abidemi[1]; Akaogu, Ijeoma[1]
[1] International Institute of Tropical Agriculture (IITA) Ibadan,P.M.B 5320 Ibadan, Oyo State, Nigeria

Vitamin A deficiency is a major health problem in sub-Saharan Africa (SSA). The maize plant has wide genetic variation for PVA and can accumulate large amount in the endosperm. Therefore, increasing PVA level in maize through breeding is a feasible and sustainable approach for alleviating its deficiency. Apart from PVA problem, maize production in SSA is constrained by Striga hermonthica parasitism, drought and low soil nitrogen (low-N). Therefore, there is need to develop and commercialize stress tolerant PVA maize in the effort to fight malnutrition in SSA. The objectives of this study were to determine the combining ability of 20 early-maturing PVA maize inbreds under contrasting environments, classify the inbreds into heterotic groups, identify testers and examine performance of inbreds in hybrid combinations. One hundred and ninety single-cross hybrid plus six yellow-endosperm hybrid checks were evaluated under drought, Striga-infested, low-N and optimal environments in Nigeria, 2016. Significant mean squares were observed for general combining ability (GCA) and specific combining ability (SCA) effects of most traits across environments. There was predominance of GCA effects for most traits, suggesting that additive gene action was more important than the non-additive. Inbreds were classified into three heterotic groups each based on GCA of multiple traits (HGCAMT) and heterotic groups' SCA and GCA of grain yield (HSGCA) methods. Inbred TZEI 129 was identified as ideal tester across environments. This tester displayed significant positive GCA for grain yield and ears per plant and significant negative GCA for Striga damage. Hence, it could be invaluable source of beneficial alleles for development of superior PVA populations and hybrids for the tropics. The AMMI biplot identified PVA hybrids TZEIOR 2 x TZEIOR 157, and TZEIOR 4 x TZEIOR 65 as outstanding in yield and stability across environments and should be commercialized in SSA.

Funding acknowledgement: International Institute of Tropical Agriculture (IITA), Ibadan-Nigeria, and the Stress Tolerant Maize for Africa (STMA), Support to Agricultural Research for Development of Strategic Crops in Africa (SARD-SC) Projects of IITA

P287

Comparative analysis of GRN and eQTL modules in maize

(submitted by Julia Kleinmanns <jakleinmanns@ucsd.edu>)
Full Author List: Kleinmanns, Julia A.[1]; Sartor, Ryan C.[1]; Briggs, Steven P.[1]
[1] Division of Biological Sciences, University of California San Diego, La Jolla, CA 92093, USA

We previously generated an integrated developmental atlas of the transcriptome, proteome, and phosphoproteome of *Zea mays* B73 and then used these three different cellular descriptions to generate transcriptome- and proteome-based networks. We profiled 23 tissues spanning vegetative and reproductive stages of maize development to generate comprehensive and integrated gene regulatory networks (GRNs).
Another study analyzed expression QTLs (eQTLs) in apices of an intermated B73xMo17 recombinant inbred line population (Li et al. 2013, Plos Genetics). In eQTL mapping transcript abundance is used as a phenotypic trait and the genomic loci controlling the transcript abundance are mapped.
To understand if GRNs and eQTLs share certain regulatory components, we compared our RNA, protein, and phosphoprotein GRN modules with the 96 identified eQTL hotspots. We identified 19 transcription factors (TFs) that are shared between the GRN and eQTL modules. Most of the TFs were exclusively found as regulators in RNA GRNs (8), non-modified protein GRNs (3) or phosphoprotein GRNs (3), whereas others were found in more than one GRN (5). An analysis of these modules will be presented.
We plan to do mutant/transgene disruption of regulator expression to test whether the regulators control expression of the target genes. Furthermore, we will do enrichment analyses and inspect our co-expression modules to predict phenotypic consequences of disrupting regulator expression.

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P288

Complex genetic architecture of maize domestication traits as explained by interaction between *teosinte branched 1* (*tb1*) and its genetic background

(submitted by Chin Jian Yang <cyang227@wisc.edu>)
Full Author List: Yang, Chin Jian[1]; Melo, Amanda A. de[1]; Yang, Liyan[2]; Doebley, John F.[1]
[1] University of Wisconsin-Madison; 425 Henry Mall; Madison, WI, USA 53706
[2] Life Science College, Shanxi Normal University, No. 1 Gongyuan St, Linfen City, Shanxi Province, 041004, China

Allelic effects of a gene are often determined by its genetic background, i.e. the allelic combination of other genes in the genome. During the domestication of maize from its progenitor teosinte, a key change at *teosinte branched 1* (*tb1*) conferred dramatic differences in both plant and ear architecture. Interestingly, previous results have shown that the phenotypic effects of *tb1* depend on multiple background loci. We mapped one of the background loci called *enhancer of tb1.2* (*etb1.2*) to a YABBY class transcription factor (*ZmYAB2.1*). *tb1* and *ZmYAB2.1* interact both at the expression and phenotypic levels in regulating ear internode length. In addition, we recently identified three additional background loci responsible for a different trait, percent staminate spikelets (STAM). Of the three background loci, two are on chromosome 1 (STAM1.1 and STAM1.2) and one is on chromosome 2 (STAM2.1). The identification of multiple background loci interacting with *tb1* represents a step towards unraveling the complexity of genetic architecture of maize domestication traits.

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

P289

Complex genetic architecture underlies maize tassel domestication

(submitted by Guanghui Xu <gh99@cau.edu.cn>)
Full Author List: Xu, Guanghui[1]; Wang, Xufeng[1]; Huang, Cheng[1]; Xu, Dingyi[1]; Li, Dan[1]; Tian, Jinge[1]; Chen, Qiuyue[1]; Wang, Chenglong[1]; Liang, Yameng[1]; Wu, Yaoyao[1]; Yang, Xiaohong[1]; Tian, Feng[1]
[1] National Maize Improvement Center, China Agricultural University, Beijing 100193, China

Maize tassels underwent profound morphological changes during maize domestication and improvement. Although a number of genes affecting maize inflorescence development have been identified, the genetic basis of the morphological changes in maize tassels since domestication is not well understood. Here, using a large population of 866 maize-teosinte BC_2S_3 recombinant inbred lines genotyped by 19,838 SNP markers, we performed high-resolution quantitative trait locus (QTL) mapping for five tassel morphological traits. We showed that the five tassel traits were associated with different genetic architecture features. Known genes for maize inflorescence development identified by mutagenesis were significantly enriched in the tassel trait QTLs, and many of these genes, including *ra1*, *bif2*, *ub2*, *zfl2* and *baf1*, showed evidence of selection. An in-depth nucleotide diversity analysis at the *bif2* locus identified strong selection signatures in the 5'-regulatory region. We also found that several known flowering time genes co-localized with tassel trait QTLs. A further association analysis indicated that the maize photoperiod gene *ZmCCT* was significantly associated with tassel size variation. Using near-isogenic lines, we narrowed down a major-effect QTL for tassel length, *qTL9-1*, to a 513-kb physical region. These results provide important insights into the genetic architecture that controls maize tassel evolution.

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P290

Composite selection mapping in three exotic maize populations: adaptation to the central U.S. Corn Belt

(submitted by Candice Gardner <candice.gardner@ars.usda.gov>)
Full Author List: Vanous, Adam E.[1]; Gardner, Candice A.[1 2]; Blanco, Michael H.[3]; Lubberstedt, Thomas L.[1]
[1] Iowa State University; 2016 Agronomy Hall; Ames, IA, 50014
[2] USDA-ARS Plant Introduction Research Unit; G212 Agronomy Hall; Ames, IA 50014
[3] USDA-ARS Retired; Encinitas, CA, 92024

The genetic and ecological factors that shaped the post-domestication spread of maize across the Americas rely heavily on adaptive flowering traits. We investigated the effects of latitudinal change in maize by using three exotic populations that have been previously adapted to the U.S. Corn Belt through artificial selection for early flowering; Tuxpeño Composite Cycle 1, 3, and 5; Suwan-1 Cycles 1, 3, and 5; and Tusón Cycles 1, 3, 5, 7, and 9. These populations and their derivative cycles of selection were genotyped using genotyping-by-sequencing technology and GBS data were analyzed using a novel Bayesian outlier test to detect genomic regions that had undergone selection.
A total of 1368 genomic regions were found, averaging 252 kb in size. Two hundred-thirty-one flowering-related candidate genes were found within these genomic regions. Many of these genes were in genomic regions identified by all three studied populations. We found that little gene diversity was lost and that the majority of selection acted upon standing variation, with less than 1% of the identified SNPs reaching fixation due to selection. Results suggest a complex involvement of many flowering related candidate genes for the adaptation of exotic germplasm to the U.S. Corn Belt. The latitudinal change that allowed for adaptation in the three studied populations fit the complex gene regulatory models that have been proposed, however the nature of many of these genes suggests that their interactions may allow for adaptation to take place in less time than the mass selection method used to adapt the three studied populations.

Funding acknowledgement: United States Department of Agriculture (USDA), Hatch Multistate Project NC-007

P291

Connection between genome divergence patterns and DNA repair systems in crops

(submitted by Jinyu Wang <jinyuw@iastate.edu>)
Full Author List: Wang, Jinyu[1]; Li, Xianran[1]; Yu, Jianming[1]
[1] Department of Agronomy, Iowa State University, Ames, IA 50011, USA

Progress of sequencing technologies has generated a huge amount of genomic sequences, which provide a great opportunity to address fundamental questions about the genome itself. By investigating DNA base composition within species, we have discovered a conserved pattern, *i.e.*, modern crops have significantly higher [AT] values than their ancestors. This consistent pattern across multiple species implies base composition can well capture the overall genome divergence assembled from millions of polymorphisms accumulated through evolution. We hypothesize that genetic components may have contributed to the genome divergence. To test this hypothesis, 100 maize genomes and 302 soybean genomes were analyzed. The well-characterized solar UV signature motifs are enriched around SNPs in both species. This indicates solar UV radiation is one of the major forces driving the divergence of plant genomes. With base-composition across polymorphic sites as the phenotype, genome-wide scans identified a set of putative candidate genes involved in DNA repair pathways. Research discoveries from this genetic mechanisms study will enrich our understanding of genome evolution.

Funding acknowledgement: National Science Foundation (NSF), Raymond F. Baker Center for Plant Breeding

P292

Contrasting regression and classification prediction methods for identifying superior maize hybrids

(submitted by Brett Burdo <burdo@wisc.edu>)
Full Author List: De Leon, Natalia[1 2]; Kaeppler, Shawn M[1 2]; Burdo, Brett L[1]
[1] Department of Agronomy, University of Wisconsin, Madison, Wisconsin, The United States of America 53706
[2] Department of Energy, Great Lakes Bioenergy Research Center, University of Wisconsin, Madison, Wisconsin, The United States of America 53706

Genomic prediction methods typically involve regression of a training set of line phenotypes on dense genetic markers. Estimated marker effects are then used to predict genotyped the value of untested lines. Recently, supervised classification methods have shown promise for genomic prediction. Classification methods train a model to best categorize a training set of lines, which and then predict the classes of new, genotyped individuals. These methods classify lines in a way that would be quite intuitive to a breeder, as class predictions could be set to a percentile for selection, or set to a culling threshold selecting only individuals that pass it. Here, we compare the performance of Support Vector Classification to that of three regression methods, Reproducing Kernel Hilbert Space (RKHS), BayesB, and ridge regression Best Linear Unbiased Prediction (rrBLUP) to accurately classify individuals to be selected into the top 20% of lines, as well as accurately classify lines as above a culling threshold. The data used to test these methods are from testcross populations of two six-line, synthetic populations, with founders chosen to represent the Stiff Stalk and Iodent heterotic groups. The lines have been genotyped at over 55,000 markers with the illumina Maize SNP50 beadchip. Hybrids were evaluated for 12 traits from 2014 to 2016.

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P293

Cyclopiazonic acid is a pathogenicity factor for *Aspergillus flavus* and a promising target for screening maize germplasm for ear rot resistance

(submitted by Subbaiah Chalivendra <schalivendra@agcenter.lsu.edu>)
Full Author List: Chalivendra, Subbaiah C[1]; DeRobertis, Catherine[1]; Chang, Perng-Kuang[2]; Damann, Kenneth E[1]
[1] Louisiana State University Ag Center, Baton Rouge, LA 70803
[2] USDA-Southern Region Research Center, New Orleans, LA 70124

Aspergillus flavus, an opportunistic pathogen, contaminates maize and other key crops with carcinogenic aflatoxins (AF). Besides AF, *A. flavus* makes many more secondary metabolites (SMs), whose toxicity in insects or vertebrates has been studied. However, the role of SMs in the invasion of plant hosts by *A. flavus* remains to be investigated. Cyclopiazonic acid (CPA), a neurotoxic SM made by *A. flavus*, is a nanomolar inhibitor of endoplasmic reticulum calcium ATPases (ECAs) and a potent inducer of cell death in plants. We hypothesized that CPA, by virtue of its cytotoxicity, may serve as a key pathogenicity factor that kills plant cells and supports the saprophytic life style of the fungus, while compromising the host defense response. This proposal was tested by two complementary approaches. A comparison of CPA levels among *A. flavus* isolates indicated that CPA may be a determinant of niche adaptation, i.e., isolates that colonize maize make more CPA than those restricted to the soil. Further, mutants in the CPA biosynthetic pathway are less virulent than their wild type parent in field inoculation assays. Additionally, genes encoding ECAs are expressed in developing maize seeds and induced by *A. flavus* infection. Building on these results, we developed a seedling assay where maize roots were exposed to CPA and cell death was measured as Evans Blue uptake. Among >40 maize inbreds screened for CPA tolerance, the publicly available AF contamination data for many lines was broadly correlated with their CPA sensitivity. In summary, our studies show that (1) CPA serves as a key pathogenicity factor that enables the saprophytic life style of *A. flavus* and (2) maize inbreds are diverse in their tolerance to CPA. Taking advantage of this natural variation, we are currently pursuing both genome-wide and candidate gene approaches to identify novel components of maize resistance to AF contamination.

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P294

Data mining and design concept to streamline the prediction-guided breeding

(submitted by Tingting Guo <tguo@iastate.edu>)
Full Author List: Guo, Tingting[1]; Yu, Xiaoqing[1]; Li, Xianran[1]; Zhang, Haozhe[2]; Zhu, Chengsong[1]; Flint-Garcia, Sherry[3]; McMullen, Michael D.[3]; Szalma, Steve[4]; Holland, James B.[4]; Wisser, Randall J.[5]; Yu, Jianming[1]
[1] Department of Agronomy, Iowa State University, Ames, IA, USA 50011
[2] Department of Statistics, Iowa State University, Ames, IA, USA 50011
[3] United States Department of Agriculture-Agricultural Research Service (USDA-ARS), and Division of Plant Sciences, University of Missouri, Columbia, Mo, USA 65211
[4] United States Department of Agriculture -Agricultural Research Service (USDA-ARS), and Department of Crop Science, North Carolina State University, Raleigh, NC, USA 27695
[5] Department of Plant and Soil Sciences, University of Delaware, Newark, DE, USA 19716

Incorporating effective tools in crop improvement is essential for agriculture to meet the sustainable food production demand. Improved technologies in generating breeding materials and collecting genotyping data facilitated the implementation of genomic selection. However, whether this process can be further improved by incorporating findings from data mining and design concept remains unclear. Here we show that representative subset selection can be applied to training set design to make genomic prediction of hybrid performance more efficient. The first method, maximization of connectedness and diversity (MaxCD), was developed from the genetic mating scheme perspective by exploring patterns in genetic relationship and phenotypic variation. The next two methods, partitioning around medoids (PAM) and fast and unique representative subset selection (FURS), were adapted from cluster analysis and graphic network analysis to the genomic prediction context. With a small training proportion, all three methods of training set design outperformed random sampling in prediction accuracy across multiple traits within a set of maize hybrids generated with diverse founders. Analyses with two larger data in wheat (early stage of hybrid breeding system) and rice (established hybrid breeding system) further demonstrated the advantage of the new methods. Two criteria, connectedness and diversity, were quantified to explain the outperformance of representative subset selection over random sampling. Unlike previous enabling technologies, genomic selection places more emphasis on how to identify training hybrids for data collection and how to achieve high prediction accuracy for a much larger set of candidate hybrids. As a result, we expect research in data mining and design optimization to offer additional helpful guidelines for plant breeding.

Funding acknowledgement: National Science Foundation (NSF)

P295

(Poster withdrawn from the program)

P296

Digging into the hidden half: Uncovering natural diversity in development of maize root system architecture

(submitted by Adam Bray <abray@danforthcenter.org>)
Full Author List: Bray, Adam[1 2]; Flint-Garcia, Sherry[2 3]; Topp, Chris[1 2]
[1] Donald Danforth Plant Science Center, St. Louis, MO, USA 63132
[2] Division of Plant Sciences, University of Missouri, Columbia, MO, USA 65211
[3] USDA-ARS Plant Genetics Research Unit, Columbia, MO, USA 65211

The abundance of genetic and above ground phenotypic diversity in maize has been the subject of many studies, however the root system remains inconspicuous. Recent advances in high-throughput root phenotyping allow for characterization of root system architecture (RSA) -- when and where a plant places its roots -- in multiple environments. RSA dictates a plant's ability to manage abiotic stresses, including lack of water and nutrients, which can have profound impacts on plant health and yield. RSA is a highly complex, quantitative trait, and little is known about the specific genes that control it. In order to understand developmental changes in RSA, the maize NAM founders were grown at the University of Missouri-Columbia Genetics Farm in 2015 and 2016. Root crowns were harvested every week starting at 4 weeks after planting through tasseling. These roots were washed and imaged for 2D RSA analysis using Digital Imaging of Root Traits (DIRT). Samples from weeks 4, 6, and tasseling were also scanned for 3D RSA using X-ray Computed Tomography (CT). This experiment was mirrored in a controlled greenhouse environment using tree pots with clay pellets to facilitate excavation of the entire root system. RSA analysis revealed developmental, time specific differences in root architecture traits as a function of natural diversity. Utilizing both 2D (digital images) and 3D (X-ray CT) analysis has provided the first steps toward a more complete and biologically relevant view of maize RSA development.

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P297

Dissecting genetic architecture of maize domestication traits and predicting candidate genes using a teosinte-maize population

(submitted by Lei Liu <lliu@cshl.edu>)
Full Author List: Liu, Lei[1 2]; He, Lili[1]; Liu, Nian[1]; Zhu, Can[1]; Hou, Rui[1]; Du, Yanfang[1]; Shen, Xiaomeng[1]; Jackson, David[1 2]; Zhang, Zuxin[1]
[1] Huazhong Agricultural University; 1 Shizishan street; Wuhan, Hubei, China 430070
[2] Cold Spring Harbor Laboratory; 1 Bungtown Road; Cold Spring Harbor, New York, USA 11724

The domestication of maize during the last ~10,000 years made a remarkable alteration from the wild species, teosinte to modern maize, for meeting human needs by selecting key genes and quantitative trait loci (QTLs). To investigate vegetative and inflorescence morphological changes during domestication, we analyzed a teosinte-maize intermated population using high-density SNP markers to dissect the genetic architecture of 13 domestication-related traits. More than two hundred common QTLs and 44 QTL-clusters were identified. Among them, a small number of common QTLs with large effect were observed, supporting previous hypotheses that a small number of major effect loci could explain a large portion of phenotype changes during domestication. However, we also found many QTLs with moderate or minor effect, which might also have a critical function for shaping the ideal plant and high yield of modern maize. Although maize has a better performance on the traits studied here, teosinte still harbored favorable alleles in some of these common QTLs, including in several major effect common QTLs. Furthermore, we integrated multiple datasets and predicted likely candidate genes underlying common QTLs, which might play importance roles for maize domestication. This study extends our understanding of the genetic basis of maize domestication, and provides important materials to dissect causal genes underlying maize domestication and to identify favorable alleles for using teosinte to enhance modern maize breeding.

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P298

Dissecting the genetics and mechanisms of the maize-*Fusarium verticillioides* pathosystem in four NAM families

(submitted by Laura Morales <lm596@cornell.edu>)
Full Author List: Morales, Laura[1]; Zila, Charles T[2]; Balint-Kurti, Peter[3 4]; Holland, James B[2 4]; Nelson, Rebecca J[1]
[1] School of Integrative Plant Science; Cornell University; Ithaca, NY, USA 14853
[2] Department of Crop Science; North Carolina State University; Raleigh, NC, USA 27695
[3] Department of Plant Pathology; North Carolina State University; Raleigh, NC, USA 27695
[4] USDA-ARS Plant Science Research Unit; Raleigh, NC, USA 27695

Fusarium verticillioides causes Fusarium ear rot (FER) and produces the mycotoxin fumonisin in maize, which can reduce yields and compromise human and animal health. Four recombinant inbred line families (CML52xB73, CML69xB73, CML333xB73, NC358xB73) from the maize nested association mapping population were grown and inoculated with *F. verticillioides* in Clayton, NC from 2012-2015. Ears were harvested at maturity and five indicators of *F. verticillioides* infection severity were measured: (1) FER, the percentage of kernels presenting symptoms, (2) symptom type, a categorization of external symptomatology, (3) kernel bulk density, indicating the relative extent of tissue degradation by the fungus, (4) grain fumonisin contamination, quantified via ELISA, and (5) grain bikaverin content, a spectrophotometric measure of the red fungal pigment. Publicly-available plant morphological and physiological traits (inflorescence architecture, flowering time, leaf angle, etc.) and GBS data were accessed from panzea.org.
Here we attempt to understand the genetic and mechanistic basis of the interaction between maize and *F. verticillioides*. Correlation analyses reveal that inflorescence traits are most closely clustered with and represent the greatest proportion of significant genetic correlations with *F. verticillioides* infection indicators. More than half of the QTL (quantitative trait loci) underlying *F. verticillioides* infection colocalize with QTL associated with other plant traits, the majority of which are inflorescence QTL. We further untangle the genetics of this multi-trait variation using multivariate models, and then assign QTL to three categories: (1) QTL that control *F. verticillioides* infection per se, (2) colocalized (due to linkage or pleiotropy) QTL for *F. verticillioides infection and other plant traits*, and (3) QTL associated with morphological/physiological traits. We compare the composition of genes within these QTL groups with GO term classification and variance partitioning to generate hypotheses about the mechanisms behind innate and morphology-mediated resistance to *F. verticillioides* infection.

P299

Distinct genetic architectures for phenotype means and plasticities in *Zea mays*

(submitted by Aaron Kusmec <amkusmec@iastate.edu>)
Full Author List: Kusmec, Aaron[1]; Srinivasan, Srikant[2 3]; Nettleton, Dan[4]; Schnable, Patrick S.[1 2]
[1] Department of Agronomy, Iowa State University, Ames, Iowa, USA 50010
[2] Plant Sciences Institute, Iowa State University, Ames, Iowa, USA 50010
[3] School of Computing and Electrical Engineering, IIT Mandi, Mandi, Himachal Pradesh, India 175005
[4] Department of Statistics, Iowa State University, Ames, Iowa, USA 50010

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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P300

Diversity of total and active nitrogen-fixing microbiome in the roots of selected grass species

(submitted by Srinivasa Chaluvadi <src@uga.edu>)
Full Author List: Chaluvadi, Srinivasa[1]; Bennetzen, Jeff[1]
[1] Department of Genetics, University of Georgia, Athens, GA 30602

Nitrogen fertilizer is critical to the growth of any crop plant. High yielding maize varieties remove 140 to 210 lbs N/acre, whereas biofuel crops such as switchgrass remove about 90 lbs of nitrogen from the soil per acre per year. Reducing the use of external nitrogen will not only help us to grow crop plants in marginal lands but also reduce the economic and environmental costs of food and biomass production. Biological nitrogen fixation (BNF) provides an alternative and eco-friendly source of nitrogen in several leguminous plants and some non-leguminous plants. Our initial studies showed that switchgrass roots were able to fix atmospheric nitrogen and that they harbor diverse populations of bacterial species that express NifH (nitrogenase) homologs. The particular NifH-expressing bacterial species vary by location and with different nitrogen fertilizer treatments. The current study was undertaken to extend our analyses and compare the composition of the total microbiome and the NifH-expressing microbiome in switchgrass, maize, sorghum, rice, and foxtail millet. Three genotypes of each species were grown under field conditions. Roots were sampled from 6-week-old plants and analyzed for NifH gene diversity in the metagenomes and the metatranscriptomes. Overall, switchgrass showed the highest abundance of nifH phylotypes in both metagenomes and metatranscriptomes, whereas maize roots showed least abundance and diversity of NifH phylotypes. Members of the Proteobacteria were routinely dominant in the roots of all the species. This poster further elaborates our results on the species-specific diversity of nitrogen-fixing microbiomes in the roots of these species.

Funding acknowledgement: Department of Energy (DOE)

P301

Effect dynamics of *qHT7.1* and *Dw3*, repulsion linked QTLs contributing to sorghum plant height heterosis

(submitted by Qi Mu <qmu@iastate.edu>)
Full Author List: Mu, Qi[1]; Underhill, Anna[1]; Li, Xin[1]; Li, Xianran[1]; Yu, Jianming[1]
[1] Department of Agronomy, Iowa State University, Ames, IA, 50011

Heterosis, the better performance of a hybrid than its parents, plays an important role in crop breeding. Pseudo-overdominance is one of four theories (dominance, overdominance, pseudo-overdominance, and epistasis) proposed to explain heterosis but with only one supporting case. Recently, we demonstrated that pseudo-overdominance explains 44% higher parent heterosis of final plant height by identifying an additional QTL, *qHT7.1*, near *Dw3* with sorghum recombinant inbred lines (RIL). Two QTLs, *qHT7.1* and *Dw3*, are 3Mb away and in repulsion phase between two parents. With two loci in repulsion phase between two inbreds, heterosis in the hybrid can appear as a single locus with an overdominance mode of inheritance. The high frequency of repulsion linked haplotype (57%) in sorghum diversity panel suggests the potential application of these two QTLs in breeding program. To better understand the effect dynamics of *qHT7.1* and *Dw3*, we measured the plant height of this RIL population across the whole growth season. The effects of *qHT7.1* and *Dw3* can be detected as earlier as the transition stage from vegetative to reproductive phase. *Dw3* reaches the highest effect around the booting stage, but the effect of *qHT7.1* keeps increasing until the final stage, which may explain previous observations that *Dw3* only affects the internode growth below flag leaf, while *qHT7.1* influences the growth of whole plant. In addition to QTL mapping, identifying the gene underlying *qHT7.1* and further phenotypic analyses will shed light on the genetic basis and molecular mechanisms of plant height and heterosis.

Funding acknowledgement: United States Department of Agriculture (USDA), Iowa State University Raymond F. Baker Center for Plant Breeding, Iowa State University Plant Sciences Institute

P302

Evaluating kernel qualities following the integration of the brown midrib 3 mutation into soft endosperm maize lines

(submitted by Bridget McFarland <bridgetm@iastate.edu>)
Full Author List: McFarland, Bridget A[1]; Scott, Paul[1]
[1] USDA-ARS Corn Insects and Crop Genetics Research Unit, Iowa State University, Ames, IA 50011

The purpose of this project is to develop soft endosperm maize lines that contain the brown midrib 3 mutation, in order to improve digestibility of silage for dairy cattle. To achieve this, we integrated the brown midrib 3 mutation (*bm3*) into soft endosperm maize lines, have evaluated the kernel hardness qualities and plan to determine the true digestibility of kernels through *in vitro* methods. *bm3* is a natural mutation occurring in maize that causes a lower lignin content and improves digestibility of vegetative tissue from corn plants in ruminant animals. Maize plants containing this recessive allele are considered valuable for silage due to their low lignin content and have even been reported to increase milk production in dairy cows. The biomass of silage is composed of half vegetative tissue and the other half comes from the grain. Maize kernels can be classified as hard or soft based on the texture of their endosperm and since cattle can more easily break down soft kernels, this allows them to access the starch, protein, fiber and minerals present inside. Our studies center around combining improved digestibility of maize containing the *bm3* mutation and the soft endosperm trait utilizing visual screens, grinding tests and true digestibility analyses of the kernel grain. After using cross-pollination to combine the traits and selection of soft endosperm lines, Near-Infrared Reflectance Spectroscopy (NIR) kernel density predictions and light box screening scores to evaluate kernel hardness were compared. Future work will need to be completed on *in vitro* digestion tests and feeding trials to confirm that this approach increases digestibility in plant biomass and kernels.

Funding acknowledgement: United States Department of Agriculture (USDA)

P303

Evaluation of nonparametric genomic selection models for predicting Single-Cross performance in maize

(submitted by Dnyaneshwar Kadam <kadam013@umn.edu>)
Full Author List: Kadam, Dnyaneshwar C[1]; Lorenz, Aaron J[1]
[1] Department of Agronomy and Plant Genetics, University of Minnesota, St. Paul, MN 55108, USA

Prediction of single-cross performance is needed to select promising single-cross combinations between parental inbred lines for field testing. Recent studies have shown the usefulness of genomic selection for the prediction the single-cross performance, but limited information is available on the potential of nonparametric genomic selection models for this purpose. The objective of this study was to evaluate three nonparametric genomic selection models -- reproducing kernel Hilbert spaces (RKHS), support vector machine (SVM) and neural network (NN) -- in comparison with genomic best linear unbiased prediction (GBLUP) for predicting the performance of single crosses. Models were compared using two separate datasets consisting of 481 and 312 single crosses made by randomly crossing recombinant inbred lines belonging to different heterotic groups. Models were built in order to predict the observed single cross performance, general combining ability (GCA) component of single cross, GCA of the parents and specific combining ability (SCA) of the cross. The predictive ability was evaluated by cross validation using four different scenarios consisting of single crosses having both tested parents (T2), either female (T1F) or male (T1M) tested parent and neither tested parents (T0). The predictive ability was greater when models were trained on GCA component for all the four scenarios. All the three nonparametric models provided increased predictive ability compared to the GBLUP for T2, T1F and T1M scenarios when the models were trained on GCA component of single cross. The predictive ability of all genomic selection models was considerably greater than phenotype based single-cross prediction, especially when one or both parents were untested. Overall, these results suggest that accuracy of genomic prediction of single crosses could be further improved by using nonparametric genomic selection models trained on the GCA component of single-cross performance. Additional research is ongoing to further test these results.

P304

Exome-seq based mapping tool to identify causal genes in maize kernel mutants

(submitted by Shangang Jia <shangang.jia@gmail.com>)
Full Author List: Jia, Shangang[1]; Li, Aixia[1]; Avoles-Kianian, Penny[2]; Kianian, Shahryar F.[2]; Zhang, Chi[3]; Holding, David[1]
[1] Department of Agronomy and Horticulture, Center for Plant Science Innovation, Beadle Center for Biotechnology, University of Nebraska, Lincoln, Nebraska 68588
[2] USDA-ARS Cereal Disease Laboratory, University of Minnesota, St. Paul, Minnesota 55108
[3] School of Biological Sciences, Center for Plant Science and Innovation, Beadle Center for Biotechnology, University of Nebraska, Lincoln, Nebraska 68588

We used gamma-irradiation to generate a population of 1788 B73 maize mutants including many with specific kernel phenotypes. Common kernel phenotypes were opaque endosperm and reduced endosperm fill. In addition to previously mapped mutants, 16 lines with kernel phenotypes, which were heritable in B73 x Mo17 F2 ears, were selected for mapping and candidate gene screening. To try to simplify and economize on our successful but separate BSR-seq and Exome-seq pipeline, we tested the ability to do BSA mapping and candidate gene screening simultaneously from exome-seq data derived from DNA from mutant and non-mutant pools (BSEx-seq). We will demonstrate the validity of this approach by exemplifying mapping and candidate genes for several new mutants. We will also compare and contrast BSEx-seq with the RNA-seq/Exome-seq approach. This study demonstrates the utility of gamma irradiation and mapping-by-sequencing to identify candidate genes for kernel deletion mutants.

Funding acknowledgement: United States Department of Agriculture (USDA)

P305

Exploiting genome by environment interaction in genomewide selection in maize

(submitted by Nick Ames <amesx083@umn.edu>)
Full Author List: Ames, Nick C[1]; Bernardo, Rex[1]; Zhong, Shengqiang[2]
[1] University of Minnesota; Department of Agronomy and Plant Genetics; St. Paul, Minnesota, 55108
[2] Monsanto; Ankeny, Iowa, United States, 50021

Genomewide selection allows a breeder to circumvent the phenotyping of candidate lines or hybrids, or reduce the extent of phenotyping of the candidates. Our objectives were to determine whether environmental effects can be predicted as a function of different environmental variables, and whether line performance can be predicted under a given set of environmental conditions. Phenotypic and marker data, which were provided by Monsanto for 969 biparental populations evaluated in 432 different environments between 2000 to 2008, included 4.2 million phenotypic data points and 11.5 million SNP data points. Along with this industry dataset, we have also obtained nine years of environmental data from National Oceanic and Atmospheric Administration database in an effort to not only select for lines with superior mean performance, but be able to select lines that maximize positive environmental interactions in specific environments. To this end, we have used a simple method of dissecting environmental effects that does not rely on crop modeling, but instead decomposes each environment into a large number of environmental components.
Correlations between observed and predicted performance for a model based on heat units and precipitation were 0.10 to 0.29 for yield, 0.14 to 0.36 for moisture, and 0.07 to 0.31 for test weight. In addition, we have used a genome by environment covariance matrix for predicting genome by environment effects. Correlations between predicted and observed line performance in a given environment were 0.25 for yield, 0.23 for moisture, and 0.36 for test weight across 22 populations. Given probable increases in environmental variability, these techniques are likely to be useful in both public and private breeding programs.

Funding acknowledgement: Monsanto

P306

Exploring the genetic basis of leaf cuticular evaporation rate in maize

(submitted by James Chamness <jchamness@gmail.com>)
Full Author List: Chamness, James[1]; Matschi, Susanne[2]; Vasquez, Miguel[2]; Kaczmar, Nick[1]; Lin, Meng[1]; Smith, Laurie[2]; Gore, Michael A[1]
[1] Plant Breeding and Genetics Section, School of Integrative Plant Science, Cornell University, Ithaca, NY 14853
[2] Cell and Developmental Biology Section, University of California San Diego, San Diego, CA 92093

Plant shoot epidermal cells extrude a hydrophobic surface layering of lipid and carbohydrate compounds known collectively as the cuticle, which confers protection to underlying tissue against pathogens and excess water loss. While major regulation of transpiration is governed by stomata, cuticular evaporation (CE) directly through the thin, hydrophobic cuticle is a significant source of water loss. It is estimated to account for 5-10% of water loss in well-watered plants during the day, but accounts for the vast majority of water loss when stomata are closed at night and under water-limited conditions. Modifying the rate of CE through selection in maize breeding programs may therefore offer a novel means to improve drought tolerance without compromising stomatal regulation of photosynthesis. In this study, the Wisconsin diversity panel of ~450 maize inbred lines was investigated for rates of leaf CE in two contrasting 2016 summer environments: Maricopa, AZ and San Diego, CA. We observed considerable natural variation for CE rates, ranging from 12.6% to 43.4% of dry weight/hour in Maricopa, and 4.2% to 33.2% of dry weight/hour in San Diego. The CE phenotype was found to have a strong genetic component, observing a broad-sense heritability of ~0.5 across environments. A genome-wide association study was initiated to identify genes controlling the CE phenotype. Concurrently, studies of leaf development, cuticle biochemical composition, and gene expression differences are ongoing to help identify candidate genes underlying GWAS signals. To better understand the role of the CE phenotype in drought tolerance, a selected subset of inbred lines that are outliers for CE rate will be evaluated for yield and its component traits under contrasting irrigation conditions in a hot, arid environment. Together, these studies will increase our understanding of the genetic basis for CE and its impact on plant productivity, with potential benefits for developing drought tolerant maize.

Funding acknowledgement: National Science Foundation (NSF)

P307

Fine-mapping a major maize domestication QTL for ear diameter

(submitted by Alessandra York <torno@wisc.edu>)
Full Author List: York, Alessandra M[1]; DeValk, Craig A[1]; Doebley, John F[1]
[1] University of Wisconsin-Madison, 425 Henry Mall, Madison, Wisconsin 53706

Maize ears are much larger in diameter and have more rows of grain than maize's ancestor, teosinte. This significant difference in ear structure makes it an essential trait to study to better understand domestication. Previously, our lab mapped domestication quantitative trait loci (QTL) in a set of maize-teosinte hybrid recombinant inbred lines (RILs) and identified a large effect QTL on the short arm of chromosome 5 for ear diameter. This QTL co-localized with a large effect QTL for kernel row number, and represents a likely domestication target. Following this work, an unsuccessful effort was made to identify the gene underlying this QTL by fine-mapping with a $BC_2 S_3$ heterogenous inbred family (HIF) derived from the RILs. Our lab also mapped this QTL using an independent set of a maize-teosinte BC_6S_6 lines segregating for this QTL region. With this set of lines, the QTL was confirmed and more narrowly mapped to a ~2.654 Mbp region. We attempted to fine-map the QTL using three of the BC_6S_6 lines to create two families that segregate for the QTL. We used known markers to identify recombination events in the region and generated a set of 170 recombinant chromosome nearly isogenic lines (RCNILs). However, once phenotyping data was collected, we found the causal region seemed to map upstream of our identified target region. We also identified another region affecting the trait on chromosome 7. Moving forward, we took a subset of RCNILs that did not segregate on chromosome 7 and were heterozygous upstream of our original target region to identify our causal region/gene responsible for differences in ear size.

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P308

Fine-mapping of a QTL associated with somatic embryogenesis and plant regeneration in maize tissue culture

(submitted by Frank McFarland <fmcfarland@wisc.edu>)
Full Author List: McFarland, Frank L[1]; Salvo, Stella A[2]; Kaeppler, Heidi F[1]
[1] Department of Agronomy, University of Wisconsin - Madison; Madison, WI 53706
[2] Monsanto; Chesterfield, MO 63067

Establishing embryogenic, regenerable tissue cultures is vital for cloning applications, genetic engineering and gene editing systems in agricultural and horticultural crops. The ability of crop plants to produce embryogenic, regenerable cultures and regenerate plants is, however, highly genotype dependent, significantly hindering progress in crop genetic research and enhancement. We are conducting research in maize aimed at deciphering the genetic mechanisms controlling differential embryogenic, regenerable response in tissue culture, which would aid in the development of improved, genotype-independent plant tissue culture and transformation systems. An inbred backcross (IB) introgression mapping population in a maize B73 background was developed from the cross of inbred maize line A188 (high embryogenic, regenerable response) with B73 (low embryogenic, regenerable response). The recurrent parent in the backcrosses, B73, is widely utilized in maize genetics research and has undergone genome sequencing. Using this initial mapping population, several QTL with significant effects on embryogenic, regenerable response were identified, with the QTL of largest effect located on the long arm of chromosome 3. Initial fine-mapping of the QTL region on chromosome 3 has been conducted via molecular marker and phenotypic analysis of lines derived from further backcrosses to B73. Currently, a genomic region 2cM in length and another of 10cM in length, located within the QTL on chromosome 3, have been shown to be associated with high embryogenic, regenerable response. Further fine-mapping population development and screening is underway to identify recombinants with reduced genomic segments that still express high embryogenic, regenerable tissue culture response. Ultimately, the goal of this project is to identify and characterize the underlying genetic factor(s) controlling genotype-dependent embryogenic, regeneration response, and to potentially produce a novel germplasm that maintains the A188-derived culture response and transformability in a B73 genetic background.

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P309

Freezing tolerance is associated with higher photosynthetic performance during chilling stress in *Tripsacum*, the sister genus of maize

(submitted by Christy Gault <cg449@cornell.edu>)
Full Author List: Gault, Christy M[1]; Jaikumar, Nikhil S[2]; Budka, Josh S[3]; Lepak, Nick K[3]; Costich, Denise[3]; Long, Stephen P[2]; Buckler, Edward S[3]
[1] Institute of Genomic Diversity, Cornell University, Ithaca, NY 14853
[2] Institute for Genomic Biology, University of Illinois, Urbana, IL 61801
[3] USDA-ARS, Ithaca, NY 14853

The maize growing season is limited by cold temperatures. Engineering a freezing-tolerant maize line would allow crops to survive severe frost events. *Tripsacum*, a freezing-tolerant relative of maize, may provide key insights in this effort. *Tripsacum* and maize diverged fewer than 1.2 million years ago and share most of their gene content (Ross-Ibarra et al., 2009). Perennial *Tripsacum* grasses exhibit freezing tolerance in northern genotypes. We are currently mapping QTL that control freezing tolerance in seedlings using bulked segregant analysis and whole genome sequencing in 54 *Tripsacum* families. In addition to surviving severe frosts, an ideal cold-tolerant maize variety would efficiently photosynthesize and accumulate biomass in cool temperatures. However, it is unknown whether C4 photosynthesis in *Tripsacum* grasses is chilling-tolerant and can help engineer chilling-tolerant maize. To address this question, freezing-tolerant and freezing-susceptible *Tripsacum* genotypes were exposed to chilling stress (10 °C day/ 5°C night) for seven days. On day 2 and day 4 of chilling stress, cut leaf sections were imaged for Fv/Fm to test for spatial variability in maximum quantum yield of PSII. Photosynthetic performance was measured before, during, and after chilling stress with a LI-COR. A two-way ANOVA revealed a significant effect of ecotype (freezing-tolerant vs. freezing-sensitive genotypes) on CO_2 uptake (A), the ratio between internal to ambient CO_2 (Ci/Ca), PhiPSII maximum efficiency (Fv'/Fm'), and maximum quantum yield of PSII (Fv/Fm). There was no significant effect of interaction between ecotype and day of experiment. These data indicate that freezing tolerance is associated with higher photosynthetic performance under chilling stress and at 25 °C. These results are compared to maize photosynthetic performance under similar levels of chilling stress.

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P310

Gene by Environment interactions of the maize ionome: lessons from NAM and the G2F project

(submitted by Greg Ziegler <gziegler@danforthcenter.org>)
Full Author List: Ziegler, Greg[2]; Roth, Jacob[1]; Wu, Di[3]; Gore, Michael[3]; Baxter, Ivan[1 2]
[1] Donald Danforth Plant Science Center; 975 N Warson Rd.; Saint Louis, MO, 63132
[2] USDA-ARS; 975 N Warson Rd.; Saint Louis, MO, 63132
[3] Cornell University; 310 Bradfield Hall; Ithaca, NY 14853;

The elemental composition (ionome) of a plant is determined by a complex interaction between genetics and growth environment. To explore the relationship between a plant's ionome, its genotype, and the environmental conditions it is grown in, we have analyzed the ionome of maize kernels in two experimental populations. The maize NAM population provides a powerful genetic tool for genome wide association analysis. We have phenotyped the NAM population in 4 grow-outs from fields in New York, Florida, North Carolina, and Puerto Rico. By exploring the differential response of the NAM lines across these locations, we have found evidence that the much of the variance in the ionomic phenotypes is due to genotype-by-environment interactions. The Genome to Fields (G2F) initiative provides a unique framework to further explore this interaction. We have analyzed the ionome of 31 varieties of maize, grown in 20 fields across the United States in two separate years. Detailed, field-level, measurements of environmental conditions provide the ability to gain a more in depth understanding of the specific factors driving ionomic variation.

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P311

Generating clonal progeny in maize

(submitted by Nina Chumak <nina.chumak@botinst.uzh.ch>)
Full Author List: Chumak, Nina[1]; Williams, Mark[2]; Brunner, Arco[1]; Fox, Tim[2]; Bernardes de Assis, Joana[1]; She, Wenjing[1]; Pasquer, Frédérique[1]; Albertsen, Marc[2]; Grossniklaus, Ueli[1]
[1] University of Zürich, Department of Plant and Microbial Biology, Zollikerstr. 107, 8008 Zürich, Switzerland
[2] DuPont Pioneer Hi-bred, P.O. Box 1000, Johnston, IA 50131-0184

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 first evidence that production of clonal individuals through seed is possible in maize.

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P312

Genetic analysis of a unique synthetic population: the Zea Synthetic

(submitted by Anna Glowinski <acs5fd@mail.missouri.edu>)
Full Author List: Glowinski, Anna C.[1]; Flint-Garcia, Sherry A.[1 2]; The, Maize Diversity Project[1 2 3 4 5 6 7]
[1] University of Missouri; Columbia, MO, 65211
[2] USDA-ARS
[3] Cornell University; Ithaca, NY, 14850
[4] North Carolina State University; Raleigh, NC, 27695
[5] University of California Davis; Davis, CA, 95616
[6] Cold Spring Harbor Laboratory; Cold Spring Harbor, NY, 11724
[7] University of Wisconsin Madison; Madison, WI, 53706

The biology of rare alleles is important to our understanding of genotype-to-phenotype relationships. Understanding the role of rare alleles can aid in the selection and development of future breeding lines. Inbreeding and selection of maize over the past 100 years has removed many deleterious alleles, but there are still rare alleles with small effects segregating in maize germplasm. However, rare alleles have not been extensively studied due to limited resolution and allelic representation issues. Therefore, a new genetic resource, the Zea Synthetic, was created utilizing both teosinte and maize. The Zea Synthetic is a randomly mated synthetic breeding population comprised of the 27 inbred Nested Association Mapping (NAM) founders and 11 geographically diverse teosinte ssp. *parviglumis* accessions. Two-thousand doubled haploids (DH) were produced from the Syn-4 generation of the Zea Synthetic to determine the contributions of allelic variants to fitness throughout the maize genome for both maize and teosinte alleles segregating in a common population. The DH have been genotyped using genotyping-by-sequencing (GBS). GBS data, combined with whole genome sequencing (WGS) of the Zea Synthetic founders, are being used in an identity-by-descent (IBD) analysis to determine which genomic regions were selected against during the DH process. Genotypic and phenotypic data are being combined in association analyses to determine phenotype and genotype relationships.

Funding acknowledgement: National Science Foundation (NSF)

P313

Genetic analysis of early-maturing maize inbreds containing genes from Zea diploperennis under Striga-infested and drought environments

(submitted by Ijeoma Akaogu <iakaogu@wacci.edu.gh>)
Full Author List: Akaogu, Ijeoma[1 2 3]; Badu-Apraku, Baffour[2]; Gracen, Vernon[1 4]; Buckler, Edward S[5 6]; Tongoona, Pangirayi[1]; Gedil, Melaku[2]; Offei, Samuel[1]; Dzidzienyo, Daniel[1]
[1] West Africa Centre of Crop Improvement, University of Ghana, PMB LG 30, Legon-Accra
[2] International Institute of Tropical Agriculture (IITA) Ibadan, P.M.B. 5320 Ibadan, Oyo State, Nigeria
[3] National Biotechnology Development Agency,(NABDA) Umaru Musa Ya'adua Way, Lugbe, Airport Road, P.M.B.5118 Abuja, Nigeria
[4] College of Agricultural and Life Science, Cornell University Ithaca, NY 14850, USA
[5] Institute of Genomic Diversity, Cornell University Ithaca NY 14850 USA
[6] United State Department of Agriculture/Agricultural Research Service, Ithaca NY 14850, USA

Infestation by Striga hermonthica (Del.) Benth and drought are two most important stresses constraining maize (Zea mays L.) production in West and Central Africa (WCA). Yield losses can reach up to 85% when the two stresses occur simultaneously in the field. One hundred and fifty hybrids derived from crosses involving 30 inbreds utilizing North Carolina Design II plus six checks were evaluated at two locations each under artificia1 Striga infestation, drought and optimal environments, 2013-2015 in Nigeria. The objective was to determine if maize hybrids containing Zea diploperennis genes could suppress Striga emergence, reduce Striga damage and produce high yield under contrasting environments. General combining ability (GCA) and specific combining ability (SCA) mean squares were significant for yield and other traits under contrasting environments. There was preponderance of GCA over SCA for yield indicating that additive gene action largely controlled the inheritance of yield. Grain yield ranged from 1.0 t ha-1 to 5.2 t ha-1 under Striga infestation, 0.5 t ha-1 to 3.6 t ha-1 under drought and 2.4 t ha-1 to 7.8 t ha-1 under optimal conditions. High yielding hybrids with reduced Striga emergence and damage were identified. Striga resistant and drought tolerant hybrids with outstanding performance across stress environments could be obtained through the accumulation of favorable alleles for stress tolerance in parental lines. Outstanding hybrids should be further tested and commercialized in Striga endemic and drought prone areas of WCA to contribute to increased maize productivity and reduced Striga seed bank in the soil. Based on this result, TZdEI 352 (Striga resistant) and TZdEI 425 (Striga susceptible) were identified and 285 F2:3 mapping population developed to identify QTLs conferring Striga resistance. The parents have the whole genome sequenced, the F2:3 are being genotyped by rAmpSeq, and the QTL mapping is in progress.

Funding acknowledgement: West Africa Center for Crop Improvement (WACCI), Alliance for a Green Revolution in Africa (AGRA), International Institute of Tropical Agriculture (IITA), Ibadan-Nigeria, The Norman Borlaug LEAP fellowship

P314

Genetic analysis of host resistance to *Setosphaeria turcica,* the causal agent of northern corn leaf blight and sorghum leaf blight

(submitted by Xiaoyue Zhang <xzhng128@illinois.edu>)
Full Author List: Zhang, Xiaoyue[1]; Mideros, Santiago[1]; Brown, Patrick[1]; Jamann, Tiffany[1]
[1] Department of Crop Sciences, University of Illinois, Urbana, IL, 61801

Northern corn leaf blight (NCLB) and sorghum leaf blight (SLB), both caused by *Setosphaeria turcica,* are important diseases of corn, and sorghum, respectively. Northern corn leaf blight is one of the top yield-limiting diseases of maize. Aside from grain loss, it can also increase susceptibility to other pathogens and decrease forage quality. Not only is this an important disease to study in order to decrease yield loss due to biotic stress, but this is an excellent system to study the evolution of pathogens on different hosts. Isolates are generally host-specific wherein maize isolates only cause disease on maize and sorghum isolates only cause disease on sorghum. While isolates are host-specific, we hypothesize that alleles that confer resistance are not host-specific. Resistance to *S. turcica* in maize has been extensively studied, yet little is known about the genetics of resistance in sorghum. We evaluated the Sorghum Conversion Panel (n= 798) to identify regions of the sorghum genome associated with resistance to *S. turcica.* The panel was screened by inoculating field-grown plants with selected sorghum *S. turcica* strains. Disease severity was rated using a percentage scale and the area under the disease progress curve was calculated. We have identified candidate genes to examine their role in defense against NCLB. Preliminary results from these studies will be presented.

P315

Genetic architecture of kernel composition in a maize natural population

(submitted by Yingni Xiao <xyn_xyn@126.com>)
Full Author List: Xiao, Yingni[1]; Hu, Shuting[1]; Li, Jiansheng[1]; Yang, Xiaohong[1]
[1] National Maize Improvement Center of China, China Agricultural University, Beijing 100193, China

The maize kernel is a staple energy source for human beings and livestock, and plays a vital role in industrial applications. As the two major composition in maize kernel, starch and protein contents are key targets in maize breeding. In this study, starch and protein contents of maize kernel were measured in three environments in a natural population which consists of 508 inbred lines. Starch and protein contents in this population ranged from 52.7% to 71.2%, and 7.8% to 15.5%, respectively. A strong negative correlation (r= -0.72) was detected between starch and protein content in the population. Using 558,630 SNPs, we performed genome-wide association studies (GWAS) to identify genetic loci associated with starch and protein contents in maize kernel. In total, 20 and 11 independent loci were significantly associated with starch and protein contents at the threshold of 1.0E−05, explaining approximately 68.0% and 35.1% of the phenotypic variation for the starch and protein. Among these loci, 4 loci were associated with both starch and protein contents, indicating pleiotropic effects on kernel composition. These results provide a further understanding of the genetic basis of maize kernel composition and will be beneficial to alter starch and protein contents in molecular breeding.

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P316

Genetic characterization of harvest and broom quality traits in broomcorn

(submitted by Jessica Bubert <jbubert2@illinois.edu>)
Full Author List: Bubert, Jessica M[1]; Brown, Patrick J[1]; Moose, Stephen P[1]
[1] Department of Crop Sciences, University of Illinois, Urbana, IL, USA 61801

Broomcorn (Sorghum vulgare var. technicum) is a type of sorghum specifically selected for its utility in the broom-making industry. Ideally, broomcorn lines have an extended panicle with the seed set limited to the very end of the panicle. The branches of the panicle would begin at the peduncle with minimal fusing of the central branches. These varieties are well suited for mechanical harvesting, after which the seed is removed and the panicle straw woven into brooms. The pedicels on the end of the straw act to capture dust particles while the thicker portion of the panicle provides support for the broom. Thus, broomcorns are an interesting example of artificial selection for specific features of grass inflorescence architecture. Broomcorn improvement at the University of Illinois over the past forty years has relied heavily on field breeding for desired harvest and broom quality traits. Recent advancements in low cost genotyping technologies have enabled gene discovery and molecular breeding in broomcorn. High throughput genotyping of all 55 broomcorn cultivars available from the USDA germplasm system generated high confidence single nucleotide polymorphisms (SNPs). Analysis of these SNPs determined the genetic differentiation of the broomcorn sorghum types relative to traditional sweet and grain sorghum lines. Additionally, phenotypes used to assess the “broominess” were collected for several hundred individuals across the 55 populations. These traits include total panicle length, proportion of panicle with seed, yield per panicle, plant height, seed color, and a qualitative measurement of central branch fusion. A genome-wide survey was used to identify loci associated with the variation observed in the broom quality and agronomic traits. Further assessment of these loci can help to elucidate the genes involved in inflorescence architecture and could aid in the improvement of broomcorn lines through the use of molecular breeding.

Funding acknowledgement: Nolan Broomcorn Trust

P317

Genetic dissection of morphological and anatomical traits using multi-parent advanced generation intercross populations of maize

(submitted by Kathryn Michel <kathrynhoemann@gmail.com>)
Full Author List: Michel, Kathryn J[1]; Burdo, Brett[1]; de Leon, Natalia[1]; Kaeppler, Shawn[1]
[1] Department of Agronomy; University of Wisconsin- Madison; Madison, WI, USA 53706

Multi-parent advanced generation intercross populations are useful tools for the dissection of complex traits in plants. The goal of this project is to provide a detailed description of the variation present in a collection of four maize double haploid (DH) populations derived from the intercrossing of six founder lines representing relevant US Corn Belt heterotic patterns including the Stiff Stalk, Iodent, and Non-Stiff Stalk groups. The fourth population is denoted Big Plant and was derived from the intercross of six large biomass-type parents. For the Stiff Stalk set, two subsets were derived, one where DHs were derived after two generations of recombination and the second subset derived after four generations of recombination.
The collection of 4292 lines was planted in two field replicates in Madison, Wisconsin during summer 2016. Data collected included plant height, ear height, number of ears per plant, days to pollen and silk, stalk diameter, tiller and node traits, and tassel traits. Three representative plants per plot were evaluated for all traits except days to pollen and silk which were measured on a plot-basis.
Iodent derived inbreds were on average more prolific than the other three populations and less variable, whereas the Non-Stiff Stalk population had the smallest number of ears per plant on average, but a larger range. The Stiff-Stalk and Big Plant populations had similar average plant height, and they were taller and more variable than the Iodent population. The Non-Stiff Stalk population had the shortest tassels with the smallest number of tassel branches of the four populations. This poster will provide detailed descriptions of the morphological assessment of this group of populations and highlight relevant associations between genotypic information and the phenotypes collected.

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P318

Genetic dissection of the maize MAMP response

(submitted by Peter Balint-Kurti <pjbalint@ncsu.edu>)
Full Author List: Zhang, Xinye[1 2]; Valdés-López, Oswaldo[3]; Arellano, Consuelo[4]; Stacey, Gary[3]; Balint-Kurti, Peter[2 5]
[1] Maize Research Institute, Sichuan Agricultural University, 211 Huimin Road, Wenjiang District, Chengdu, Sichuan 611130, China
[2] Department of Entomology and Plant Pathology, North Carolina State University, Raleigh, NC 27695, USA
[3] Divisions of Plant Science and Biochemistry, University of Missouri, Columbia, MO 65211, USA
[4] Statistics Department, North Carolina State University, Raleigh, NC 27695, USA
[5] U.S. Department of Agriculture, Agricultural Research Service (USDA-ARS) Plant Science Research Unit, Raleigh, NC, USA

Microbe-associated molecular patterns (MAMPs) are highly conserved molecules commonly found in microbes which can be recognized by plant pattern recognition receptors (PRRs). Recognition triggers a suite of responses including production of reactive oxygen species (ROS) and nitric oxide (NO) and expression changes of defense related genes. In this study, we used two well-studied MAMPs (flg22 and chitooctaose) to challenge different maize lines to determine whether there was variation in the level of responses to these MAMPs, to dissect the genetic basis underlying that variation and to understand the relationship between MAMP response and quantitative disease resistance (QDR). Naturally-occurring quantitative variation in ROS, NO production and defense genes expression levels triggered by MAMPs was observed. A major quantitative traits locus (QTL) associated with variation in the ROS production response to both flg22 and chitooctaose was identified on chromosome 2 in a recombinant inbred line (RIL) population derived from the maize inbred lines B73 and CML228. Minor QTL associated with variation in the flg22 ROS response were identified on chromosomes 1 and 4. Comparison of these results with data previously obtained for variation in QDR and the defense response in the same RIL population did not provide any evidence for a common genetic basis controlling variation in these traits.

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P319

Genetic dosage analysis of alleles conferring quantitative disease resistance to *Setosphaeria turcica,* the causal agent of northern corn leaf blight

(submitted by Mercy Kabahuma <kabahuma@iastate.edu>)
Full Author List: Kabahuma, Mercy, K[1 2]; Posekany, Tesia, S[1 2]; Studt, Jacob, E[2]; Kuehne, Grace, N[2]; Lopez, Miriam, D[2 3]; Lauter, Nick[1 2 3]
[1] Genetics and Genomics Graduate Program, Iowa State University, Ames, IA, 50011
[2] Department of Plant Pathology and Microbiology, Iowa State University, Ames, IA, 50011
[3] USDA-ARS Corn Insects and Crop Genetics Research Unit, Iowa State University, Ames, IA, 50011

Plant diseases account for more than 40% of crop losses. Breeding for disease resistance is among the most effective ways to protect crops. However, the absence or instability of qualitative resistance mechanisms in many crop-pathogen systems has necessitated a shift toward research on Quantitative Disease Resistance (QDR) mechanisms. Quantitative genetic studies in maize are often conducted on inbred populations, including those performed on disease traits. Since corn is cultivated as a hybrid crop, it is also important to conduct studies using populations of hybrids because allele dosage and genetic environment differences may limit the extensibility of results obtained from research using inbreds alone. To address QTL dosage and genotypic context effects for traits that measure resistance to *Setosphaeria turcica,* we conducted two years of replicated field trials on a set of 200 Intermated B73xMo17 Doubled Haploid lines (IBMDHLs) and their two semi-hybrid progeny populations derived from backcrossing to each respective parent. All 600 lines plus check genotypes were manually inoculated at the V6-V7 stage and again at the V7-V8 stage using inoculum made from the IA-01 isolate of *Setosphaeria turcica.* For each of the 2400 plots in the experiment, disease severity was scored on plots of 25 plants once per week for the two-month period following manual inoculation. Here we report on comparisons of quantitative disease progression outcomes across the three populations and between the two years to address questions concerning the genetic modes of action and strengths of effect for the quantitative disease resistance loci we have identified.

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

P320

Genetic mapping and introgression of leaf angle QTL in maize

(submitted by Matthew Dzievit <mdzievit@iastate.edu>)
Full Author List: Dzievit, Matthew J.[1]; Li, Xianran[1]; Yu, Jianming[1]
[1] Department of Agronomy, Iowa State University, Ames, IA, USA

Improved canopy architecture is one of the ways maize hybrids have adapted to higher plant densities. Modern hybrids have been moving increasingly towards upright leaves, which assist in distributing light more effectively in the canopy. This study is being conducted to further the understanding of maize canopy architecture, specifically to identify genetic components controlling leaf angle (LA) variation. PHW30 was identified as having an upright LA (75.9°), whereas B73 (63.1°) and Mo17 (55.7°) have a flatter LA relative to PHW30. Four reciprocal bi-parental populations (upright x flat) were developed, and LAs for the F_2 progeny were measured. Genotyping by sequencing was used to genotype all four F_2 populations. Quantitative trait loci (QTL) mapping was conducted using inclusive composite interval mapping for each F_2 population. We identified 2 QTLs for the Mo17 populations and 4 QTLs for the B73 populations, which explained 40.1% and 40.4% of the variation, respectively. The QTL on chromosome 1 was present in both populations, and we observed repulsion linkage between two QTLs on chromosome 3 for the B73 population. Concurrently, selected progeny from the F_2 populations were used to generate double haploid lines, and the favorable LA QTL from PHW30 are being backcrossed from PHW30 into B73 and Mo17 to further assess the effect of LA variation. Selection of these QTL can be used to develop maize inbred lines with ideal canopy architectures and help improve yield under high planting densities.

Funding acknowledgement: National Science Foundation (NSF)

P321

Genetic mapping of QTL for maize leaf width combining IF2 and RIL populations

(submitted by Ruixiang Liu <maize2008@hotmail.com>)
Full Author List: Liu, Ruixiang[1]; Zheng, Fei[1]; Meng, Qingchang[1]; Cui, Yakun[1]; Kong, Lingjie[1]; Chen, Yanping[1]; Yuan, Jianhua[1]; Lübberstedt, Thomas[2]
[1] Institute of Food Crops, Jiangsu Academy of Agricultural Sciences, Nanjing, Jiangsu Province, China, 210014
[2] Iowa State University, Ames, IA, USA, 50011

Leaf width is an important component of plant architecture, which affects light capture during photosynthesis and wind circulation under dense planting conditions. To extend our understanding of the genetic mechanisms involved in the width of leaves at different positions, a comprehensive evaluation using RIL and IF2 populations was performed followed by a meta-analysis. Fifty-nine QTL, 21 heterotic loci and 122 pairs of significant digenic epistatic interactions associated with leaf width at different positions below the tassel were detected. The individual effects of QTL ranged from 2.12% to 15.60% of the observed phenotypic variation with ten QTL showing effects above 10%. The initial QTL were integrated into 11 mQTL through a meta-analysis. Our results suggest that leaf width at different positions may be affected by several of the same mQTL, and also regulated by many different mQTL. We found that most other significant interactions are of the Non-QTL/Non-QTL and Non-QTL/QTL types and that leaf width heterosis is mostly due to overdominance and dominance. These results provide useful information for breeding high density tolerant inbred lines and hybrid cultivars, and for using marker-assisted selection for important mQTL.

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P322

Genome wide association analysis of *Striga hermonthica* resistance in a sorghum MAGIC population

(submitted by Megan Fenton <fentonm@purdue.edu>)
Full Author List: Fenton, Megan E.[1]; Ejeta, Gebisa[1]
[1] Purdue University, Department of Agronomy, West Lafayette, IN 47907

Sorghum (*Sorghum bicolor* [L.] Moench) is currently grown on 44.2 million hectares, ranking sorghum as the fifth most important cereal globally. Sorghum is a climate resistant cereal that is a crucial staple food crop for a number of developing countries. Approximately 78.8% of the sorghum being produced is grown in regions of the world, primarily Sub-Saharan Africa and India, where *Striga* species are an established and intensifying pest. The *Striga* genus consists of approximately 30 obligate root parasite species. However, only *Striga hermonthica* and *Striga asiatica* afflict sorghum and maize. These *Striga* species only germinate in response to chemical stimulants produced by the sorghum host plant. Once the *Striga* germinates it attaches to the root system and deprives the host of water and nutrients, resulting in a drastic yield reduction that can result in a total crop loss. The objectives of this research are to (1) determine the genetic mechanisms that confer resistance to *Striga* in sorghum and (2) implement genomic prediction modeling to assist in selecting *Striga* resistant sorghum lines using solely genotypic information. These objectives will be achieved using the PP37 sorghum population of 1,000 recombinant inbred lines that was constructed through six generations random mating of 25 sorghum lines that have varying degrees of *Striga* resistance and agronomic performance. The PP37 population is a unique multi-parent advanced generation intercross (MAGIC) population that has been genotyped with genotyping-by-sequencing (GBS). Phenotypic data on *Striga* resistance and agronomic performance indicators was collected in Northwestern Ethiopia where *Striga* infestations are at epidemic levels. This genotypic and phenotypic information will be utilized in a genome wide association study (GWAS) to determine the regions of the genome that influence *Striga* resistance. This data will also be used to construct a training population to develop a genomic prediction model that will potentially allow resistant sorghum lines to be selected from sorghum lines that only have genotypic information available.

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P323

Genome-wide association study and genomic prediction models for mineral levels in maize grain

(submitted by Di Wu <dw524@cornell.edu>)
Full Author List: Wu, Di[1]; Stangoulis, James[2]; Rocheford, Torbert[3]; Gore, Michael A.[1]
[1] Plant Breeding and Genetics Section, School of Integrative Plant Science, Cornell University, Ithaca, NY, USA 14853
[2] School of Biological Sciences, Flinders University, Bedford Park, South Australia, Australia
[3] Department of Agronomy, Purdue University, West Lafayette, IN, USA 47907

Collectively impacting more than two billion people worldwide, mineral nutrient deficiencies, principally in iron and zinc, are global health problems that predominantly occur in the developing world. Biofortification, or the genetic improvement of nutritional content in the consumed portion of crops such as maize grain, offers an approach that is both sustainable and cost-effective to alleviate and prevent mineral deficiencies in humans. The maize Ames inbred panel, with a population size of nearly 2,000 lines and capturing a high level of allelic diversity, provides a powerful platform for studying the genetic basis of mineral concentration in grain. Inductively coupled plasma-mass spectrometry (ICP-MS) was used to generate grain mineral profiles consisting of nine elements, including iron and zinc, for Ames inbred lines grown at a single location (Indiana) in 2012, with a second replicate from 2013 yet to be analyzed. In the first step to identify causal genes for these nine mineral traits, a genome-wide association study was conducted using ~338,000 SNP markers scored on the Ames inbred panel. Several hundred SNP markers were significant for one or more mineral traits; of these, 42 and 68 SNPs were detected for Fe and Zn, respectively, at a genome-wide FDR of 5%. To provide insight into the feasibility of genomic selection as a method for breeding of these traits, ridge regression best linear unbiased prediction models based on the full SNP marker data set generated prediction accuracies ranging from 0.24 to 0.49 for the nine elements. Notably, Fe and Zn had moderately high prediction accuracies of 0.49 and 0.38, respectively. The knowledge produced from this study will ultimately help to accelerate marker-based breeding efforts for improved Fe and Zn grain concentration in maize biofortification breeding programs around the world.

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P324

Genome-wide association study dissects the genomic treasures of elite maize inbred lines

(submitted by Yingjie Xiao <shanren0179@163.com>)
Full Author List: Xiao, Yingjie[1]; Wang, Xiaqing[1]; Qiao, Feng[1]; Yang, Wenyu[1]; Liu, Haijun[1]; Zhang, Ruyang[2]; Luo, Jingyun[1]; Niu, Luyao[1]; Song, Wei[2]; Li, Chunhui[2]; Zhao, Yanxin[2]; Meng, Yijiang[3]; Zhao, Jiuran[2]; Yan, Jianbing[1]
[1] National Key Laboratory of Crop Genetic Improvement, Huazhong Agricultural University, Wuhan, 430070, China.
[2] Maize Research Center, Beijing Academy of Agriculture & Forestry Sciences, Beijing Key Laboratory of Maize DNA Fingerprinting and Molecular Breeding, Beijing 100097, China.
[3] College of Life Science, Hebei Agricultural University, Baoding, 071001, China.

Maize (*Zea Mays* L.) is one of the most important crops globally for food, feed and fuel. Since 1980s, the maize heterotic groups and its representative elite inbred lines were frequently used in single-cross hybrid breeding worldwide, but the underlying basis lack of understanding. We here propose a maize synthetic population, tailor-made for genetics and breeding, with thoroughly intercross across 24 maize elite inbred lines in China followed by sufficiently inbreeding (single-seed decent for over six generations). We uncovered over 50,000,000 SNPs and 2,800,000 indel polymorphisms and collected multi-environmental 20 phenotypes including flowering, agronomic and yield traits. We reconstructed the identity-by-descent (IBD) map for any progeny in synthetic population, reflecting a high-resolution reshuffle across 24 parental genomes. We performed genome-wide association study (GWAS) at the single-variant and IBD scales, successfully identifying important known genes and many new loci, which jointly explained over 75% total genetic variance for all traits in average. We found the enrichment and dilution of specific parental compositions play vital roles in the short-term phenotype selection. The most contributing parents varied with trait per se and overall performance, probably caused by genetic heterogeneity and synergistic effects (or pleictropism). We discuss the potential roles of complementation of heterotic information and key IBD regions in breeding by design.

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P325

Genome-wide association study using whole-genome sequencing rapidly identifies new genes influencing agronomic traits in rice

(submitted by Masanori Yamasaki <yamasakim@tiger.kobe-u.ac.jp>)
Full Author List: Yamasaki, Masanori[1]; Yano, Kenji[2]; Maeda, Michihiro[1]; Yoshida, Shinya[3]; Kitano, Hidemi[4]; Hirano, Ko[4]; Tamiya, Gen[5]; Doi, Kazuyuki[6]; Matsuoka, Makoto[4]
[1] Food Resources Education and Research Center, Graduate School of Agricultural Science, Kobe University, Kasai, Hyogo, Japan 675-2103
[2] Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo, Japan
[3] Hyogo Prefectural Research Center for Agriculture, Forestry and Fisheries, Kasai, Hyogo, Japan
[4] Bioscience and Biotechnology Center, Nagoya University, Nagoya, Japan
[5] Tohoku Medical Megabank Organization, Tohoku University, Sendai, Japan
[6] Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya, Japan

A genome-wide association study (GWAS) can be a powerful tool for the identification of genes associated with agronomic traits in crop species, but it is often prevented by population structure and the large extent of linkage disequilibrium. In the present study, we performed identification of agronomically important genes in rice using GWAS based on whole-genome sequencing, followed by the screening of candidate genes based on the estimated effect of nucleotide polymorphisms. Using this approach, we identified novel genes associated with agronomic traits. Several genes were undetectable by standard SNP association analysis, but were detected using gene-based association analysis. This study provides fundamental insights relevant to the rapid identification of genes associated with agronomic traits using standard GWAS and nested association mapping (NAM) GWAS, and will accelerate future efforts aimed at the genetics and breeding in not only rice but also maize.

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P326

Genomewide recurrent selection for Fusarium ear rot and Fumonisin resistance in maize

(submitted by Thiago Marino <tpmarino@ncsu.edu>)
Full Author List: Marino, Thiago[1]; Manching, Heather[2]; Wisser, Randall[2]; Holland, James[1 3]
[1] North Carolina State University, Department of Crop and Soil Sciences, Raleigh, NC 27695-7620
[2] University of Delaware, Department of Plant and Soil Sciences, Newark, DE 19716
[3] USDA-ARS, Plant Science Research Unit, Raleigh, NC 27695-7620

Fusarium ear rot (FER) of maize is caused by *Fusarium verticillioides*, which produces fumonisin (FUM), a mycotoxin linked to human and animal health risks. Sources of resistance to FER have been identified, but the resistance is polygenic and difficult to incorporate into elite hybrids. Extensive field trials, laborious inoculation, and expensive antibody assays are required to reliably assess resistances to FER and FUM contamination in breeding populations. Genomic selection (GS) could improve the efficiency of breeding for these complex disease resistance traits by training selection models on a subset of a breeding population and applying them to a larger sample of genotyped but untested lines from the population. This can increase the number of lines screened beyond the limits of field screening capacity, effectively increasing selection intensity. To evaluate the potential utility of GS in an ongoing maize breeding program, we called 6131 SNPs on 508 $S_{0:1}$ families from an advanced generation of a recurrent selection program using low coverage sequence. A training set of 263 $S_{0:1}$ lines was evaluated for FER and FUM at three locations during two years. The remaining 245 $S_{0:1}$ lines were evaluated as an independent validation set in a subsequent year. Preliminary results indicate high accuracy between predicted and true genetic values for FER (0.59) and FUM (0.65) for an independent set of lines tested in the same year as the training set. The 20 most resistant lines predicted on the training set were less affected by *Fusarium verticillioides* compared to the population mean and the mean of the most susceptible lines. These results provide evidence that GS is a very promising breeding strategy for Fusarium resistance. We are developing strategies to implement GS for additional generations of selection before retraining the model.

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P327

Genomic estimation of complex traits in archaeological maize reveals ancient adaptation to temperate North America

(submitted by Kelly Swarts <kelly.swarts@tuebingen.mpg.de>)
Full Author List: Swarts, Kelly[1 2]; Gutaker, Rafal M.[2]; Schuenemann, Verena J.[3 4]; Benz, Bruce[5]; Blake, Michael[6]; Bukowski, Robert[7]; Holland, James[8]; Kruse-Peeples, Melissa[9]; Lepak, Nick[10]; Matson, R.G.[6]; Prim, Lynda[9]; Romay, M. Cinta[11]; Ross-Ibarra, Jeffrey[12]; Sanchez-Gonzalez, Jose de Jesus[13]; Schmidt, Chris[9]; Sofro, Evan[9]; Krause, Johannes[3 14]; Weigel, Detlef[15]; Buckler, Edward S.[1 10]; Burbano, Hernán A.[2]
[1] Dep. of Plant Breeding and Genetics, 175 Biotechnology Bldg., Cornell Univ., Ithaca, NY 14853 USA
[2] Research Group for Ancient Genomics and Evolution, Department of Molecular Biology, Max Planck Institute for Developmental Biology, Spemannstr. 35, 72076 Tübingen, Germany
[3] Institute of Archaeological Sciences, University of Tuebingen, Tuebingen 72076, Germany
[4] Senckenberg Center for Human Evolution and Paleoenvironment, University of Tübingen, Tübingen 72076, Germany
[5] Department of Biology, Schollmaier Science and Technology Rm 109 , Texas Wesleyan University, Forth Worth, TX 76105 USA, Fort Worth, TX 76105
[6] Department of Anthropology, Vancouver Campus, 6303 NW Marine Drive, V6T 1Z1, Vancouver, BC, Canada
[7] Bioinformatics Facility, Institute of Biotechnology, Cornell University, Ithaca, 14853, NY USA
[8] USDA-ARS and Department of Crop and Soil Sciences, Box 7620, North Carolina State University, Raleigh, 27695-7620, NC, USA
[9] Native Seeds/SEARCH 3584 E. River Rd., Tucson, 85718, AZ USA
[10] USDA-ARS, Ithaca, 14853, NY, USA
[11] Genomic Diversity Facility, 175 Institute of Biotechnology, Cornell University, Ithaca, 14853, NY USA
[12] Dept. of Plant Sciences, Center for Population Biology, and Genome Center, University of California, One Shields Ave, Davis, 95616, CA, USA
[13] Centro Universitario de Ciencias Biológicas y Agropecuarias, Universidad de Guadalajara, Zapopan, Jalisco CP45110, Mexico
[14] Department of Archaeogenetics, Max -Planck -Institute for the Science of Human History für Menschheitsgeschichte, Kahlaische Strasse 10, 07745 Jena, Germany
[15] Department of Molecular Biology, Max Planck Institute for Developmental Biology, Spemannstr. 35, 72076 Tübingen, Germany

People introduced maize (*Zea mays* ssp. *mays*) to the southwestern US by 4,000 years ago, quickly establishing maize agriculture in the lowland deserts while adoption was delayed in the temperate uplands for 2,000 years. Because reduced flowering time characterizes modern temperate maize, we test for early flowering in the earliest established upland agriculture. Turkey Pen Shelter, at 1830m in southeastern Utah, is one of the earliest established agricultural sites in the temperate Southwest. We sequenced fifteen 1,800-year old archaeological maize cobs from Turkey Pen Shelter with high endogenous content to 5-20X coverage, allowing genotyping on HapMap 3.21 variants. An additional 1018 GBS-genotyped teosinte and landrace individuals enriched for the Southwest US and North Mexico cover the parameter space of tropical and temperate adapted landraces, allowing us to genetically situate Turkey Pen maize within modern temperate Southwestern landraces. A subset of 110 Southwest US/N. Mexican individuals, representing 80 accessions, were crossed to a common tester and the progeny evaluated for DTA and DTS in 9 replicate/environments (H = 0.89 and 0.9, respectively). Using the diverse Ames inbred panel to train genomic prediction models, which had a prediction accuracy of 0.72 in the phenotyped Southwestern landraces, we confidently predicted that Turkey Pen maize flowered comparably early to modern temperate lines, and would have been marginally adapted in Utah. Our results suggested that temperate adaptation drove modern population differentiation and was selected in situ from ancient standing variation. Reliance on standing genetic variation highlights the importance of germplasm maintenance in light of unprecedented climate change.

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P328

Genotype-by-environment interactions affecting heterosis in maize

(submitted by Zhi Li <lixx5447@umn.edu>)
Full Author List: Li, Zhi[1]; Coffey, Lisa[2]; White, Michael[3]; Keiter, Brad[2]; Garfin, Jacob[1]; Miller, Nathan D.[4]; Spalding, Edgar[4]; Leon, Natalia de[3]; Kaeppler, Shawn M.[3]; Schnable, Patrick S.[2]; Springer, Nathan M.[5]; Hirsch, Candice N.[1]
[1] Department of Agronomy and Plant Genetics, University of Minnesota, Saint Paul, MN 55108
[2] Department of Agronomy, Iowa State University, Ames, IA 50011
[3] Department of Agronomy, University of Wisconsin, Madison, WI 53706
[4] Department of Botany, University of Wisconsin, Madison, WI 53706
[5] Department of Plant and Microbial Biology, University of Minnesota, Saint Paul, MN 55108

The manifestation of heterosis, the phenomena in which the offspring of two inbred parents exhibits superior phenotypic characteristics, can be influenced by environmental factors. By definition, the magnitude of heterosis is directly determined by the performance of the hybrid and its parents. When maize hybrids and parental genotypes respond differently to environmental stimuli, interaction between heterosis and environment is observed. In this study we monitored 25 traits ranging from vegetative growth (plant height at nine developmental stages, flowering time, leaf number, etc.) to yield components (ear and cob weight, kernel height, grain weight, etc.) in a set of 47 maize hybrids and their inbred parents that were grown in 16 different environments each with varying levels of average productivity. The magnitude and rank of better-parent heterosis (BPH) varied across traits and environments. Across the traits, a higher within plot variance was observed for inbreds compared to hybrids. A similar trend was also observed for variance across environments, however for most traits the difference was not significant. The magnitude of correlations of BPH and. hybrid performance and BPH and. better parent performance was consistent but in opposite directions across the 25 traits. These results indicate that the genotype-by-environment interaction for heterosis is comparably driven by changes in the hybrid and the better parent performance. Additionally, relatively weak correlations were observed for BPH between traits at early developmental stages and traits later in development. This study highlights the complexities of the interaction of heterosis and the environment and the difficulty in linking “omic” data from a single sample to a range of performances in different environments and across various traits.

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P329

Genotypic diversity in the responses of yield and yield components to elevated ozone of diverse inbred and hybrid maize

(submitted by Lorena Rios-Acosta <lrios@illinois.edu>)
Full Author List: Ríos-Acosta, Lorena[1]; Erice, Gorka[1]; Kendzior, Matt[1]; Lewis, Mark[1]; Mulcrone, Jessica[1]; Resano-Goizueta, Inés[1]; Thompson, Ben[1]; Tomaz, Tiago[1]; Barrios-Perez, Ilse[1]; Montes, Chris[1]; Sorgini, Crystal[1]; Wedow, Jessica[1]; Brown, Patrick J[1]; McIntyre, Lauren[2]; Ainsworth, Elizabeth A[3]; Leakey, Andrew DB[1]
[1] University of Illinois at Urbana-Champaign, Urbana, IL
[2] University of Florida, Gainsville, FL
[3] USDA ARS, Urbana, IL

Current tropospheric ozone concentrations ([O_3]), an important air pollutant, are phytotoxic and detrimental to crop yield causing significant losses of ~14-26 billion in 4 of the world’s major crops. Until recent years, it was believed that agricultural and economically important C_4 plants, such as maize, were not significantly affected by O_3. Therefore we have a limited knowledge of the genetic and physiological basis of maize yield loss due to oxidative stress caused by O_3.This project evaluated variation in the effects of elevated ozone (100ppb) on yield and yield components (ear number, individual kernel weight or kernel number) across diverse genotypes of inbred and hybrid maize during 3 growing seasons at the Free Air Concentration Enrichment (FACE) site in Champaign, IL. In 2014, 52 inbred lines representing the extremes of O_3 sensitivity were tested in addition to 26 hybrids. In 2015, 10 inbred lines were retested in addition to 8 hybrid lines. Primary kernel mass (yield) was, on average, significantly lower in inbred and hybrid lines for 2014 and 2015 respectively. While some lines were sensitive to yield loss (up to -76% in inbreds and -26% in hybrids) others were highly tolerant of growth at elevated O_3. Yield loss was primarily driven by decreased kernel number in inbreds, and by decreased individual kernel mass in hybrid genotypes. Inbred genotypes, B73 and Mo17 were identified as O_3 tolerant and O_3 sensitive, respectively. Therefore in 2016, 100 B73-Mo17 NILs (50 B-NILs containing Mo17 introgressions in a B73 background and 50 M-NILs containing B73 introgression in a Mo17 background)were evaluated for the response of yield traits to elevated O_3 to perform quantitative trait locus (QTL) discovery.

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

P330

Harnessing genomic variability to reduce stalk lodging in maize

(submitted by Esperanza Shenstone <shensto2@illinois.edu>)
Full Author List: Shenstone, Esperanza[1]; Jamann, Tiffany[1]; Lipka, Alexander E.[1]
[1] Department of Crop Science, University of Illinois, Urbana, Illinois 61801

Stalk lodging is defined as the breakage of a corn stalk below the ear. Every year, the maize industry is faced with yield loss due to stalk lodging. The abundance of new genomic information available has increased the ability of computational tools to study the genetic basis of agricultural traits. This project will use statistical approaches to rigorously quantify the genomic underpinnings of stalk lodging in maize using data collected at the University of Illinois, as well as data from multiple locations across the United States as part of the Genomes 2 Fields project. We have found stalk lodging to follow a non-normal distribution and to be a low heritability trait, both of which pose a challenge for analyzing the relationship between genotype and phenotype. To address these issues, multiple statistical approaches will be explored, such as including additional covariates in our model and using non-parametric approaches. The goals of this project is to be able to conduct a genome-wide association study (GWAS) and evaluate the ability of genomic selection to predict stalk lodging. Depending on the underlying genetic architecture of lodging and marker density, it is possible that a GWAS will detect moderate- to large- effect loci that are associated with lodging tolerance. The same set of markers will also be used to create genomic selection models, which will enable an assessment of the ability to breed for lodging tolerance in maize based on marker information alone. The predictive ability of these models will be assessed through cross validation. The results from this study will underscore breeding efforts for lodging tolerance in maize by determining if substantial reductions in breeding cycle time can be realized through the utilization of genomic markers.

Funding acknowledgement: United States Department of Agriculture (USDA)

P331

Heterosis in maize: intermated recombinant inbred lines and their immortalized F2 reveal (i) QTLs with dominant effects but no overdominance and (ii) a large contribution of epistasis for grain yield

(submitted by Alain Charcosset <charcos@moulon.inra.fr>)
Full Author List: Ben Sadoun, Sarah[1]; Fievet, Julie[1]; Mary-Huard, Tristan[1]; Nicolas, Stéphane[1]; Falque, Matthieu[1]; Gallais, André[1]; Laborde, Jacques[2]; Moreau, Laurence[1]; Charcosset, Alain[1]
[1] INRA - Université Paris-Sud - CNRS - AgroParisTech; Ferme du Moulon; 91190 Gif-sur-Yvette; France
[2] INRA; 2297 route de l'INRA; 40390 Saint Matin de Hinx;France

Heterosis is a major phenomenon in the living world. It is defined as the superiority of a hybrid over its parents. To identify genetic factors implied in heterosis in maize we studied a population composed of 184 highly recombinant inbred lines and 312 hybrids with a genetic structure of immortalized F2.
We confirmed that the magnitude of heterosis was variable depending on the trait studied. The average performance of parents appeared highly correlated to the performance of hybrids. The slope for yield was significantly higher than one, suggesting the contribution of epistatic effects. The high number of recombination events lead to a reduced variation of the genetic distance between the parents, which probably explains the limited relationship that was observed between the heterozygosity rate and the degree of heterosis. This high number of recombination events also had an impact on the detection of QTLs (Quantitative Trait Loci) and HL (heterotic Loci. The 28 QTLs detected and 3 HL had a moderate dominant effect and no apparent overdominance. This supports the hypothesis that the overdominant QTLs that were observed in other studies were probably related to the linkage between QTLs with dominant effects. Analysis of epistasis between QTLs is under investigation.

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P332

High density bin mapping in a sorghum RIL population [*S. propinquum* × *S. bicolor* (Tx7000)] for comparative analyses with foxtail millet and maize to determine the genetic architecture of tillering

(submitted by Rajanikanth Govindarajulu <rajini28m@gmail.com>)
Full Author List: Govindarajulu, Rajanikanth[1]; Henderson, Ashley[1]; Ramachandran, Dhanushya[1]; Chaluvadi, Srinivasa R[2]; Bennetzen, Jeffery[2]; Hawkins, Jennifer H[1]
[1] Department of Biology, West Virginia University, Morgantown, WV 26506, USA
[2] Department of Genetics, University of Georgia, Athens, GA 30602, USA

Tillers are vegetative branches that grow at or near the ground level and play an important role in plant biomass production and grain yield. Comparative analyses described here can delineate the factors that regulate the number, size, and fertility of tillers among diverse taxa. In this study, we use whole-genome based high density bin mapping to identify tillering QTL in a sorghum recombinant inbred mapping population derived from a cross between a wild highly-tillered relative and a domesticated cultivar with suppressed tillering [*S. propinquum* × *S. bicolor* (Tx7000)], and compare our results to that of foxtail millet and maize to investigate the gene networks involved in growth and development of tillers in panicoid grasses. Phenotypes from both greenhouse and field experiments indicate significant genotypic effects on tillering. We sequenced whole genomes of 193 RILs and the parental lines at ca. 2X and 18X depths, respectively. Using a sliding window approach, 362,265 SNPs were grouped into 1,315 bins that were used as markers in QTL mapping. In total, 849 segregating bin markers were retained, producing a map length of 930 cM with an average interval of 1.1 cM between bins. Five significant QTL were detected for four traits, two of which control tillering and are located on chromosomes 1 (59 -64 Mb) and 6 (24 -26 Mb). We then compared all sorghum genes within the tillering QTL window to *Setaria* using synmap in CoGe to identify syntenic QTL regions, and found sorghum genes overlapping with markers linked to tillering on *Setaria* chromosomes 5, 7 and 9.

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P333

HiLo: Evolutionary genetics of highland adaptation in maize and teosinte

(submitted by Jeffrey Ross-Ibarra <rossibarra@ucdavis.edu>)
Full Author List: Coop, Graham[1]; Flint-Garcia, Sherry[2]; Hufford, Matthew B[3]; Rellán-Álvarez, Rubén[4]; Ross-Ibarra, Jeffrey[5]; Runcie, Daniel E[6]; Sawers, Ruairdh J[7]
[1] Center for Population Biolgoy and Dept of Evolution and Ecology, University of California, Davis, CA 95616
[2] U.S. Department of Agriculture-Agricultural Research Service, Columbia, MO 65211, USA
[3] Dept of Ecology, Evolution, and Organismal Biology, Iowa State University
[4] Laboratorio Nacional de Genómica para la Biodiversidad, Irapuato, México
[5] Center for Population Biology, Genome Center, and Dept of Plant Sciences, University of California, Davis, CA 95616
[6] Dept of Plant Sciences, University of California, Davis, CA 95616
[7] Laboratorio Nacional de Genómica para la Biodiversidad, Irapuato, México

The genetic basis of plant adaptation to their local environments remains poorly characterized, despite its relevance to climate change and crop improvement. We are investigating the genome-wide underpinnings of local adaptation in wild and domesticated populations of maize to high elevation environments. We will first identify quantitative trait loci for highland adaptation traits using mapping populations developed from Mexican and South American maize, a landrace association mapping panel, a naturally admixed population of highland and lowland teosinte and two populations of doubled-haploid introgression lines donated by industry collaborators. These populations will allow for comparison of the genetic architecture and effect sizes of highland traits in distinct geographical regions, across elevations, and in both teosinte and maize. Second, we will investigate population genetic evidence of selection through studies of adaptive introgression from maize into teosinte and adaptive divergence in gene expression between lowland- and highland-adapted maize. Finally, the functional consequences of a putatively adaptive inversion polymorphism identified in highland landraces will be characterized through phenotypic and transcriptomic evaluation of introgression lines.

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P334

Identification of genetic determinants of maize tassel structure

(submitted by Yan Zhou <yzhou86@iastate.edu>)
Full Author List: Zhou, Yan[1]; Srinivasan, Srikant[1 2]; Kusmec, Aaron[1]; McNinch, Colton[1]; Chung, Yong Suk[1]; Schnable, Patrick S.[1]
[1] Department of Agronomy, Iowa State University, Ames, Iowa, USA 50011
[2] Current Address: School of Computing and Electrical Engineering, IIT Mandi, Mandi, Himachal Pradesh, India, 175005

Structural variation in inflorescence traits of cereal crops can influence yield. To identify genetic factors that contribute to the structural variation of inflorescences, we phenotyped maize tassels via a field-based imaging platform. Tassels were classified as “open” or “closed” based on whether or not the central spike was occluded by tassel branches. A genome-wide association study (GWAS) conducted for this trait using 3, 336 NAM RILs identified multiple distinct trait associated SNPs (TAS). Mu insertion alleles of several loci adjacent to TAS identified exhibited altered tassel morphology, suggesting a role for these genes in tassel architecture.

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P335

Identifying deleterious mutations in maize

(submitted by Fei Lu <fl262@cornell.edu>)
Full Author List: Lu, Fei[1]; Romay, Maria Cinta[1]; Bukowski, Robert[1]; Johnson, Lynn[1]; Buckler, Edward[1 2]
[1] Institute for Genomic Diversity, Cornell University, Ithaca, New York, United States of America
[2] United States Department of Agriculture/Agricultural Research Service, Ithaca, New York, United States of America

Deleterious mutations are constantly reducing fitness of all species by disrupting genes and functional elements across the genome. They contribute to fitness, inbreeding depression, and, likely, heterosis in maize through complementation. Due to natural selection, deleterious mutations are rare in population. Traditional quantitative genetic approaches suffer from limited resolution (QTL mapping) or insufficient allelic replication (GWAS) to allow accurate effect estimates of these rare deleterious mutations. Motivated by identifying deleterious alleles and purging them from population, we sequenced 380 maize inbred lines over 10X coverage and conducted variants discovery from the data set. Variant effect estimating tools, including Genomic Evolutionary Rate Profiling (GERP) and Sorting Tolerant From Intolerant (SIFT), were used to identify 115,170 deleterious mutations in maize. We found deleterious mutations have lower allele frequency than neutral ones. Deleterious mutations are enriched in centromeric regions where recombination rate is low (65% increase), suggesting recombination is a key factor purging deleterious mutations. The lowly expressed/translated, tissue specific expressed/translated genes generally have more deleterious mutations (Walley et al. 2016. Science). We found pollen expressed genes exhibited the least amount of deleterious mutations when compared with genes expressed in other tissues (Chettoor et al. 2014. Genome Biology), indicating gametophyte stage and pollen competition are efficient to purge deleterious mutations. In addition, more deleterious mutations were found in teosintes and landraces than elite breeding lines. Although intensive breeding effort has been effective in removing deleterious mutations in maize, the remaining deleterious mutations significantly change the phenotype of 18 traits in the maize nested association mapping (NAM) populations, showing their large effect on phenotypic variance of maize. These identified deleterious mutations will be incorporated into genomic prediction for multiple traits. It is anticipated that modeling deleterious mutations will enhance current genomic selection approaches and accelerate breeding process of maize and many other crops.

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P336

Influence of leaf rolling on canopy light environment and yield response to drought revealed by hemispherical imaging in Setaria

(submitted by Darshi Banan <banan2@illinois.edu>)
Full Author List: Banan, Darshi[1]; Paul, Rachel[1]; Feldman, Max[2]; Holmes, Mark[1]; Schlake, Hannah[1]; Baxter, Ivan[2 3]; Leakey, Andrew[1]
[1] University of Illinois Urbana Champaign 1402 IGB 1206 W Gregory Dr Urbana, IL 61801 USA
[2] Donald Danforth Plant Science Center 975 North Warson Road St. Louis, MO 63132
[3] USDA-ARS, Donald Danforth Plant Science Center 975 North Warson Road St. Louis, MO 63132

Tuning plant architectural responses to abiotic signals has the potential to improve next generation biofuel crop productivity on marginal lands and in the face of climate change stressors. In cereals, leaf rolling as a reversible means to adjust the canopy microenvironment is an under-improved behavior that has the potential to influence yield responses to drought stress. Hemispherical canopy imaging was used to estimate two canopy properties using the model C4 grass system Setaria viridis: Plant Area Index (PAI), the amount of plant tissue area per unit ground area; and Global Site Factor (GSF), the proportion of light that passes through a canopy. A RIL mapping experiment showed that PAI has strong phenotypic correlation with total above-ground biomass and QTL for PAI co-localize with QTL for traits describing biomass productivity. A GWAS drought experiment showed that diurnal changes in the canopy light environment measured by GSF correspond with diurnal changes in plot-level leaf rolling score and directly measured leaf-level leaf rolling angle. PAI evaluated from dawn hemispherical images was used as a mid-season yield estimate. The percent treatment differences of dawn PAI correlated with the percent time differences of drought-stressed plot-level leaf rolling score (r2=0.58) and leaf-level leaf rolling angle (r2=0.59). Initial GWAS analysis detected seven SNPs related to plot-level leaf rolling score. Results demonstrate that leaf rolling is a good predictor of yield responses to drought. Future work will use a greenhouse grown GWAS population to investigate the genetic and cellular basis of leaf rolling. Leaf rolling score will be evaluated with greater temporal resolution and microscopy will be used to characterize the number and size of bulliform cells, the osmotic motors that drive leaf rolling. Mapping results will be used to determine how regions related to rolling correspond to homologs from other cereal crops already described in the literature.

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P337

Integration of physiology and genetic load with genome wide prediction for drought tolerant sorghum

(submitted by Ravi Valluru <rv285@cornell.edu>)
Full Author List: Valluru, Ravi[1*]; Brown, Patrick J.[2]; Leakey, Andrew D.B.[2]; Gore, Michael A.[1]; Buckler, Edward S.[1]
[1] Institute for Genomic Diversity, Cornell University, Ithaca, NY 14853
[2] Institute for Genomic Biology, University of Illinois Urbana-Champaign, Urbana, IL 61801

*Correspondence and Presenter: rv285@cornell.edu

Genomic selection (GS) can be more effective than conventional phenotype-based approaches in hybrid breeding. In the Genome Wide Prediction (GWP) models used in GS, genetic effects are normally modeled either as an additive or additive plus dominant fashion that improved the predictive ability of complex traits. The actual realism of a trait physiology can be however different than this simple supposition where genomes associated with complex traits carry an uncertain amount of gene mutational load across many metabolic pathways. Such a mutational load may substantially contribute to the total genetic variation of quantitative traits. Current GWP models do not fully account for mutational load background, as allele polymorphisms causing mutational load exists almost in a heterozygous fashion and hence are nonlinearly correlated with phenotypes. Therefore, an integration of metabolic pathway-based gene mutational load knowledge into GWP models might determine the total genetic contribution to, and improve the prediction accuracy of, complex traits such as heterosis of hybrids. As a part of ARPA-E funded project 'Water Efficient Sorghum Technologies (WEST)', our work focuses on (1) estimation of gene mutational load, (2) estimation of metabolic pathway-specific mutational dosage, and (3) an integration of the pathway-specific mutational load into GWP models to improve the prediction of heterosis for enhanced water use efficiency (WUE) of energy sorghum hybrids. The project employs a set of sorghum transgenics affected in stomatal and leaf developmental traits to predict natural variation for WUE traits. In addition, we also dissect and predict yield component traits as modeling multivariates improve predictive power than individually targeting traits. Finally, the project incorporates cis-regulated expression networks into hybrid prediction models. An integration of genetic load-by-pathway and cis-regulated expression networks into GWP models would constitute an elegant set of approaches towards hybrid prediction models.

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P338

Intra-specific variation for carbon sequestration potential in maize

(submitted by Sarah Hill-Skinner <shillski@iastate.edu>)
Full Author List: Hill-Skinner, Sarah[1]; Zhang, Wei[2]; Castellano, Michael[3]; Nettleton, Dan[4]; Schnable, Patrick S.[1]
[1] Department of Agronomy, Iowa State University, Roy J. Carver Co-Lab, Ames, IA 50011-3650, USA
[2] Department of Statistics, Iowa State University, 3414 Snedecor Hall, Ames, IA 50011-1210, USA
[3] Department of Agronomy, Iowa State University, 2104L Agronomy Hall, Ames, IA 50011-3150, USA
[4] Department of Statistics, Iowa State University, 2115 Snedecor Hall, Ames, IA 50011-1210, USA

Multiple scientific bodies such as the Intergovernmental Panel on Climate Change (IPCC) have concluded that rising concentrations of atmospheric CO_2 are driving climate change that will result in significant negative impacts on the environment and economy. The deployment of crop varieties bred for carbon sequestration has been proposed by the IPCC and The National Academy of Sciences as a potential method to sequester atmospheric CO_2. Although inter-specific variation for carbon sequestration potential has been documented, it is not known whether crops exhibit sufficient intra-specific variation for this trait to enable the breeding of "carbon sequestering" varieties. In this study, we examine carbon sequestration potential of a large, diverse population of maize lines via analysis of carbon emission over time. Included in our study are differences in carbon emission patterns, variation across environments, and relation of emission to important biochemical traits.

P339

Intragenic meiotic recombination generates novel gene expression patterns

(submitted by Alina Ott <aott@iastate.edu>)
Full Author List: Liu, Sanzhen[1]; Schnable, James C.[2]; Ott, Alina[3]; Yeh, Eddy[3]; Springer, Nathan M.[4]; Yu, Jianming[3]; Muehlbauer, Gary[5]; Timmermans, Marja C. P.[6]; Scanlon, Michael J.[7]; Schnable, Patrick S.[3]
[1] Department of Plant Pathology, Kansas State University, Manhattan, KS 66506
[2] Department of Agriculture and Horticulture, University of Nebraska-Lincoln, Lincoln, NE 68588
[3] Department of Agronomy, Iowa State University, Ames, IA 50011-3605
[4] Microbial and Plant Genomics Institute, Department of Plant Biology, University of Minnesota, Saint Paul, MN 55108
[5] Department of Agronomy and Plant Genetics, University of Minnesota, Saint Paul, MN 55108
[6] Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724
[7] Department of Plant Biology, Cornell University, Ithaca, NY 14853

The shuffling of sequences among different haplotypes by meiotic recombination creates new genetic variation. In particular, intragenic recombination has the potential to combine portions of different alleles to create novel protein variants. We hypothesize that intragenic recombination could also create novel expression patterns. We performed RNA-Sequencing on 105 maize recombined inbred lines derived from two maize inbred lines to understand how the genome-wide patterns of meiotic recombination and intragenic recombination affect gene expression. In total, 7,574 crossovers and 922 non-crossover recombinants were observed. 793 recombinant alleles of 561 distinct genes were created via intragenic crossover. Importantly, we validated our hypothesis by identifying recombinant alleles exhibiting non-parental expression patterns. Furthermore, recombined hotspot genes are more likely to be syntenic with sorghum, and recombination is more prevalent in syntenic regions

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P340

Intraspecific variation of residual heterozygosity and its utility for quantitative genetics studies in maize

(submitted by Nannan Liu <935019928@qq.com>)
Full Author List: Liu, Nannan[1]; Liu, Jianxiao[2]; Li, Wenqiang[1]; Pan, Qingchun[1]; Wang, Hong[1]; Zhan, Wei[1]; Liu, Jie[1]; Yang, Xiaohong[3]; Yan, Jianbing[1]; Xiao, Yingjie[1]
[1] National Key Laboratory of Crop Genetic Improvement, Huazhong Agricultural University, Wuhan 430070, China.
[2] College of Informatics, Huazhong Agricultural University, Wuhan 430070, China.
[3] National Maize Improvement Center of China, Beijing Key Laboratory of Crop Genetic Improvement, China Agricultural University, Beijing 100193, China.

Residual heterozygosity (RH) in advanced inbred progenies of plants benefits quantitative trait locus (QTL) mapping studies. However, knowledge of factors affecting its genome-wide distribution remains limited. We collected a set of 2196 maize heterogeneous inbred family (HIF) lines from 12 recombinant inbred line (RIL) populations that were genotyped using a Maize50K SNP chip, which were collapsed to 18,615 unique RH intervals ranging from 505 to 2095 intervals per population with average coverage of 94.8% of the maize genome. In each line, the average size of RH intervals was about 58.7Mb ranging from 7.2 to 74.1Mb, and there was averagely 8.6 intervals ranging from 1.8 to 14 per line. On average, a given RH region was presented in 5 different individuals within a given population. Seven RH hotspots where RH segments enriched in the genome were identified. The RH patterns varied significantly across populations, presumably reflecting differences in genetic backgrounds and 8 QTLs were identified affecting heterozygosity level in the RH hotspots. The potential use of the HIF library to efficiently fine map QTL was assessed by one major QTL for kernel tocopherol content, down to approximately ≤ 1Mb-resolution, based on publicly available information for the 12 populations. The library of HIF lines offers insights into the RH landscape and its intraspecific variation and provides a useful resource for QTL cloning of agronomically important traits in maize.

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P341

Investigating kinship and heterotic relationships of expired maize plant variety protection lines

(submitted by Mike White <mrwhite4@wisc.edu>)
Full Author List: White, Mike R[1]; Mazaheri, Mona[1]; Gage, Joseph[1]; Mikel, Mark[2]; deLeon, Natalia[1]; Kaeppler, Shawn[1]
[1] University of Wisconsin, Madison, WI 53706
[2] University of Illinois, Urbana, IL 61801

The Plant Variety Protection (PVP) Act of 1970 gives breeders exclusive right to distinct sexually or tuber propagated plant varieties for a period of 20 years. Upon expiration of the certificate, PVP maize inbred lines become publicly available and are released through the North Central Regional Plant Introduction Station. Expired-PVP maize inbred lines can be an excellent source of elite germplasm to enhance or initiate a breeding program. The use of expired-PVP maize inbred lines in a breeding program is challenging because pedigrees are often ambiguous, making it difficult to utilize lines in appropriate heterotic pools. Empirical observations in the literature and from our group indicate that the concept of heterotic groups is much more complex than the canonical Stiff Stalk vs non Stiff Stalk grouping.
Relationships among 240 expired-PVP and public reference inbred lines were evaluated using neighbor joining with approximately 430K SNPs derived from RNA-sequencing. To further assess relationships of expired-PVP lines, an admixture for K groups was performed and identity by state matrices were generated to visualize diversity within this set. Using cross validation scores, K= 8 yielded the best fit for the expired-PVP panel. The admixture results placed a traditional inbred founder in each of the eight groups. These include B14, B37, B73, Oh43, Mo17, PH207, PHWG5, and PHGG7. Finally, a chromosome identity analysis was performed to visualize chromosomal parental contributions to progeny in more detail. Together, these analyses provide strong evidence of shared and private alleles among heterotic pools and private sector originator breeding programs.
Utilization of relatedness information of expired-PVP lines can help to better categorize lines with previously unknown pedigree information to expedite the utilization of expired-PVP material in a breeding program.

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P342

Investigating the genetics of selection response for flowering time in a multi-environment parallel selection experiment

(submitted by Heather Manching <hcorn@udel.edu>)
Full Author List: Manching, Heather K[1]; Dumas, Michael[1]; Wills, David[2 3]; de Leon, Natalia[4]; Flint-Garcia, Sherry[2 3]; Holland, James[5 6]; Lauter, Nick[7 8]; Murray, Seth[9]; Weldekidan, Teclemarian[1]; Xu, Wenwei[10]; Wisser, Randall J[1]
[1] Department of Plant and Soil Sciences; University of Delaware; Newark, DE, USA 19716
[2] USDA-ARS; Columbia, MO, USA 65211
[3] Division of Plant Sciences; University of Missouri; Columbia, Missouri, USA 65211
[4] Department of Agronomy; University of Wisconsin; Madison, Wisconsin, USA 53706
[5] USDA-ARS; Raleigh, NC, USA 27695
[6] Department of Crop Science; North Carolina State University; Raleigh, NC, USA 27695
[7] USDA-ARS; Ames, IA, USA 50011
[8] Interdepartmental Genetics Graduate Program; Iowa State University; Ames, IA, USA 50011
[9] Department of Soil and Crop Sciences; Texas A&M University; College Station, TX, USA 77845
[10] Lubbock Research and Extension Center, Texas A&M AgriLife Research, Lubbock, TX, USA

Genetic diversity is key to adaptation and breeding for crop improvement. In maize, there is a large amount of natural variation within tropically-adapted germplasm, which is a critical resource for breeding programs. However, capitalizing on these exotic germplasm is challenging, because maladaptive phenotypes for some traits can mask favorable phenotypes for other traits (of interest) when individuals are evaluated in their non-adapted, but targeted, environment. Therefore, understanding the genomic response to selection for traits relevant to adaptation, such as flowering time, is crucial for tapping into exotic sources of diversity . We created a TROPICal Synthetic (TROPICS) population of maize from seven tropical inbred lines and utilized a parallel selection experiment for early flowering time across a latitudinal transect (eight locations spanning from Puerto Rico to Wisconsin) for two generations. During each generation of selection at each location, individuals were sampled from the extreme tails in flowering time (384 individuals per tail from populations of ~10,000 individuals) which are now being genotyped. Following selection, remnant seed from all 16 of the TROPICS selected populations and the original founder population were evaluated in replicated field trials at all eight of the original selection sites, providing insight into genotype-by-environment interactions relevant to local versus broad adaptation. Using extreme mapping (thus far, completed for four locations and two generations) several highly significant associations have been detected that were consistent across generations in the same location, but were not present across all environments. Additionally, for locations that exhibited the greatest response to selection based on phenotypic analysis in the reciprocal transplant trial, there appeared to be a much greater signal for genomic response. Data analysis for extreme mapping of our TROPICS parallel selection experiment is ongoing and the latest results from the study will be reported.

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P343

Key loci playing pleiotropy roles during maize domestication identified by QTL analysis of leaf-root-ionome in TeoNILs

(submitted by Zhengbin Liu <zliu@danforthcenter.org>)
Full Author List: Liu, Zhengbin[1]; Floro, Eric[1]; Ziegler, Greg[1 2]; Baxter, Ivan[1 2]; Flint-Garcia, Sherry[2 3]; Topp, Chris[1]
[1] Donald Danforth Plant Science Center, St. Louis, Missouri, 63132
[2] Plant Genetics Research Unit, USDA–Agricultural Research Service, Columbia, Missouri, 65211
[3] Division of Plant Science, University of Missouri, Columbia, Missouri, 65211

Root traits were under indirect selection while the aboveground shoot traits were selected directly during maize domestication and artificial selection, though it has never been regarded as a breeding target due to the difficulties of selection in crop breeding programs. One unexplored hypothesis underlying this phenomenon is the long-standing point, the root-shoot communication. Here we explore this hypothesis with an integrative analysis of the genetic architectures of maize shoot, root and ionomic traits through a quantitative genetics approach. With high throughput phenotyping techniques, shovelomics (Trachsel et al., 2011), ionomics (Salt et al., 2008), and high throughput GBS (genotyping by sequencing) data (Elshire et al., 2011), QTLs controlling root system architecture, shoot and ionome traits were identified in a maize TeoNILs population. Unsurprisingly, a majority of QTLs were found to have small to moderate allele effects while fewer had large effects. Several co-localized loci, or QTL-hotspots, that play pleiotropic roles during maize domestication were identified. One identified hotspot indicated that level of nutrients P and K could potentially affect the development of root tip diameter (TD) and leaf angle (LA). The co-localizations may be attributable to shoot-root communications or genetically co-regulated changes in those traits. Together, our results not only shed light on the complex, genetic basis for differences in root system, ionome, and leaf traits between natural populations but also confirmed the existence of shoot root communications at quantitative genetics level, at least in maize.

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P344

Mapping oxidative stress response QTL in B73 - Mo17 NILs

(submitted by Crystal A. Sorgini <sorgini2@illinois.edu>)
Full Author List: Sorgini, Crystal A.[1]; Brown, Patrick J.[1]; Ainsworth, Elizabeth A.[1 2]; Leakey, Andrew D.B.[1]
[1] University of Illinois at Urbana-Champaign, IL
[2] USDA ARS, Urbana-IL

Tropospheric ozone (O3) is an air pollutant that costs billions of dollars in global crop losses. Few studies have investigated the effects of elevated ozone on C4 plant development. The goal of this study was to identify maize loci associated with variation in O3-induced oxidative stress. Based on preliminary data showing that Mo17 was more susceptible to O3 than B73, we screened 100 B73-Mo17 NILs (50 B-NILs containing Mo17 introgressions in a B73 background and 50 M-NILs containing B73 introgression in a Mo17 background) in the field under ambient (~40 ppb) and elevated (~100 ppb) O3 concentrations using the FACE (Free Air Concentration Enrichment) facility at the University of Illinois. Leaf damage was scored on a 0-9 scale at two time points: 5th true leaf at 43 DAP ("early") and on the 2nd leaf below the whorl at 90 DAP ("late"). Leaf damage was significantly higher in elevated O3 rings in both B73 (late measurement) and Mo17 (early & late measurements). Mo17 was more sensitive than B73 in the early measurement, and some Mo17 NILs were much more sensitive than Mo17. In M-NILs, significant leaf damage QTL were identified on chromosome 2 for both the early (~161 Mb) and late (~128 Mb) measurements. In each case, B73 introgressions into Mo17 in this region made NILs more susceptible. Growth chamber studies are being performed on selected NILs to assess the feasibility of QTL fine-mapping in a controlled environment.

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P345

Mapping QTL for spontaneous haploid genome doubling (SHGD) under selective genotyping

(submitted by Jiaojiao Ren <renjiaojiao789@sina.com>)
Full Author List: Ren, Jiaojiao[1 2]; Wu, Penghao[3]; Trampe, Benjamin[1]; Verzegnazzi, Anderson[1]; Frei, Urusla K.[1]; Chen, Shaojiang[2]; Lübberstedt, Thomas[1]
[1] Department of Agronomy, Iowa State University, Ames, Iowa, USA 50011
[2] National Maize Improvement Center, China Agricultural University, Beijing, China 100193
[3] College of Agronomy, Xinjiang Agricultural University, Urumqi, China 830052

Production of doubled haploid (DH) lines is a very important step in modern maize breeding. Studies on the genetic basis of spontaneous haploid genome doubling (SHGD) is important for DH production. However, it is still poorly understood, so in this study, we conducted a QTL analysis for SHGD in selected haploid populations. 'GF1' with high SHGD and 'GF3', 'GF5', and 'BS39' with poor SHGD, were selected to develop 'F1' haploid mapping populations. Of the 1078 haploids derived from 'GF1/GF3', 61 haploids with the best performance of male fertility and 30 male sterile haploids were selected to form a mapping population and screened by a 6K SNP chip. These selected individuals with extreme phenotypic values increased the statistical power of QTL detection. By comparing the frequencies of a marker allele in the fertile haploids and sterile haploids, a total of five QTL were identified on chromosomes 1, 3, 5, 6, and 9 by chi-squared tests ($p<0.05$). Composite interval mapping (CIM) was performed with those 91 haploids, three QTL were detected on chromosomes 5, 6, and 9. All three QTL were detected by both methods. The QTL *qshgd1*, *qshgd2*, and *qshgd3* explained 17.5, 10.1, and 8.7% of the total phenotypic variation, respectively. Except *qshgd3*, the sources of haploid genome doubling alleles were derived from 'GF1'. To confirm the major QTL *qshgd1*, allele frequencies at the markers within the *qshgd1* region were tested in haploids of crosses 'GF1/GF5' and 'GF1/BS39' with the best performance of male fertility. The allele frequencies showed a significant deviation from the expected 1:1 ratio, supporting the transferability of QTL alleles increasing SHGD into other backgrounds. A QTL study based on haploids from 250 'GF1/GF3' F2:3 families will be conducted in 2017 for validation and extension of this preliminary study.

P346

Marker assisted selection of in vivo maize haploid inducers suited for automatic haploid identification

(submitted by Chenxu Liu <liuchenxusdau@126.com>)
Full Author List: Liu, Chenxu[1]; Dong, Xin[1]; Tian, Xiaolong[1]; Li, Wei[1]; Chen, Baojian[1]; Wang, Lele[1]; Zhong, Yu[1]; Chen, Chen[1]; Chen, Shaojiang[1]
[1] National Maize Improvement Center of China, China Agricultural University, Beijing, China 100193

Double haploid breeding (DH) had been widely used in modern maize breeding. Maternal in vivo haploid induction by Stock6 derived inducers was one of the most important and effective method. Haploid induction rate (HIR) and haploid kernels identification were two key steps affecting haploid production efficiency. In recent years, haploid kernel identification method based on kernel oil content (KOC) had been developed, and was proved to be an efficiency way. As the result, to select maize haploid inducers with high HIR and high KOC was important for improving maize haploid production efficiency. In this research, an inducer CAU2 with HIR~10% and a high oil material Beijing high oil population (BHO) with KOC~15%-18% was used as two parents, and used for breeding of inducers with high oil content and high HIR.
In F2 generation, marker assistant selection of the most important QTL-qhir1 was conducted, individuals with homozygous qhir1 from CAU2 was selected and selfed to acquire F3 progenies. From F2 generation, individual were pollinated to at least three ears of ZhengDan958 to test HIR, for kernels of each self-pollinated ear, KOC were determined with nuclear magnetic resonance (NMR). Individuals with relative high HIR and kernels in the corresponding ears with relative high oil content were selected, and used for acquiring next generation. After 5-generation selection, In the F6 generation, 2 genetically stable families were acquired with HIR>10% and KOC>10%. The families could be used for automatically identification of haploids with high efficiency.

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P347

Morphological characterization and genetic dissection of maize yield component traits collected by image analysis

(submitted by Celeste Falcon <cfalcon@wisc.edu>)
Full Author List: Falcon, Celeste M.[1]; Haase, Nicholas J.[2]; Miller, Nathan D.[3]; Hirsch, Candice N.[4]; Yandeau-Nelson, Marna D.[5]; Spalding, Edgar P.[3]; Kaeppler, Shawn M.[1]; de Leon, Natalia[1]
[1] Department of Agronomy, University of Wisconsin-Madison, 1575 Linden Drive, Madison, WI 53706
[2] DuPont Pioneer, 19456 MN HWY 22, Mankato, MN 56001
[3] Department of Botany, University of Wisconsin-Madison, 430 Lincoln Drive, Madison, WI 5370
[4] Department of Agronomy and Plant Genetics, University of Minnesota, St. Paul, Minnesota 55108
[5] Department of Genetics, Development and Cell Biology, Iowa State University, Ames, IA 50011

Studying yield component traits allows plant breeders and geneticists to further dissect the genetic architecture of yield and to exploit this knowledge to help meet the world's growing energy demands. In this study, we aimed to identify genomic regions that contribute to yield component traits in maize using genome-wide association analysis of 837 diverse maize inbred lines, which were genotyped with approximately 430,000 RNA-seq based single nucleotide polymorphism (SNP) markers. The lines were evaluated in replicated trials in 2013 and 2014 in Arlington, WI. A subset of 500 of the lines were evaluated at two field sites in St. Paul, MN and one site in Waseca, MN in 2015 and at one site in St. Paul, MN and another in Ames, IA in 2016. An automated analysis pipeline was used to generate images of ears, cobs, and kernels collected from three representative plants per plot and to computationally extract nine phenotypic measurements from these images: ear width, ear length, kernel row number, kernels per row, kernel weight, kernel depth, kernel width, kernel area, and kernel thickness. Additionally, principal component (PC) analysis was used to develop quantitative proxies for ear shape. Strong positive correlations were evident among several traits including kernel weight/kernel area and ear width/kernel area, while other traits—including ear length/kernels per row and ear PC1/kernel depth—exhibited strong negative correlations. Trait value ranges were between 0.88- and 2.43-fold, and trait heritability ranged from 0.65 to 0.93. Multiple genomic associations were identified for every trait except ear PC2. The image analysis method used here is more holistic and about five times faster than manual measurements, enabling more accurate phenotyping of larger populations. Additionally, the significantly associated SNP markers help to locate genomic regions contributing to yield and could be used in breeding approaches to develop higher yielding cultivars.

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P348

Natural variation for food grade corn quality traits relevant to chip processing

(submitted by Mark Holmes <holme616@umn.edu>)
Full Author List: Holmes, Mark[1]; Annor, George[2]; Jia, Haiyan[3]; Gusmini, Gabe[3]; Bernardo, Rex[1]; Hirsch, Candice[1]
[1] Department of Agronomy and Plant Genetics, University of Minnesota, St. Paul, MN 55108
[2] Department of Food Science and Nutrition, University of Minnesota, St. Paul, MN 55108
[3] PepsiCo, St. Paul, MN 55108; Gabe Gusmini and Haiyan Jia are employees of PepsiCo, Inc. The views expressed in this presentation are those of the authors and do not necessarily reflect the position or policy of PepsiCo Inc.

In the United States over 90 million acres of corn are grown annually, but less than three percent of that acreage is devoted to food grade corn and a fraction of that for corn chip production. Due to the relatively small size of the chip food grade corn market, little is known about the compositional traits necessary to meet quality specifications for the post-harvest processing chain. However, it is hypothesized that the concentration and composition of starches and proteins in the endosperm are important factors contributing to the ability of a hybrid to pass through a commercial production line. To better understand the natural variation that exists for compositional traits related to food grade corn production, a set of 100 inbred lines was selected based on genetic and spectral diversity for deep compositional characterization. These lines were assayed in triplicate for protein, oil, reducing sugars, fiber, and ash content. Substantial variation in trait ranges was observed for many of the traits, for example, crude protein varied between 8.4% and 14.8% within this set of 100 lines. Wet chemistry on these lines is currently being used to develop NIR equations that will be used to survey a larger panel of lines grown over multiple environments to relate compositional trait measurements with empirical cook test results.

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P349

Networks of genetic control for maize grain carotenoid levels in the US-NAM panel

(submitted by Christine Diepenbrock <chd45@cornell.edu>)
Full Author List: Diepenbrock, Christine H.[1]; Kandianis, Catherine B.[1 2]; Lipka, Alexander E.[3]; Magallanes-Lundback, Maria[2]; Vaillancourt, Brieanne[4]; Gongora-Castillo, Elsa[4]; Wallace, Jason G.[3]; Cepela, Jason[4]; Mesberg, Alex[2]; Bradbury, Peter J.[3 5]; Ilut, Daniel C.[1]; Mateos-Hernandez, Maria[6]; Owens, Brenda F.[6]; Tiede, Tyler[6]; Buckler, Edward S.[1 3 5]; Buell, C. Robin[4]; Rocheford, Torbert[6]; Gore, Michael A.[1]; DellaPenna, Dean[2]
[1] Cornell University, Plant Breeding and Genetics Section, School of Integrative Plant Science, Ithaca, NY 14853.
[2] Michigan State University, Department of Biochemistry and Molecular Biology, East Lansing, MI 48824.
[3] Cornell University, Institute for Genomic Diversity, Ithaca, NY 14853.
[4] Michigan State University, Department of Plant Biology, East Lansing, MI 48824.
[5] United States Department of Agriculture-Agricultural Research Service (ARS), Robert W. Holley Center for Agriculture and Health, Ithaca, NY 14853.
[6] Purdue University, Department of Agronomy, West Lafayette, IN 47907.

A more comprehensive dissection of the loci governing variation in carotenoid (provitamin A) accumulation and retention is needed to improve the levels and balance of these essential nutrients in major cereal crops. Our joint linkage-genome wide association study of maize grain carotenoid levels in the 5000-line US nested association mapping (US-NAM) population revealed that the majority of identified quantitative trait loci (QTL) were underpinned by causal genes with *a priori* roles in carotenoid synthesis and degradation, including prenyl group synthesis. Six of the 10 identified *a priori* genes were expression QTL (eQTL), showing strong correlations between gene expression levels and QTL allelic effect estimates. Most of these eQTL also had high correlation of QTL allelic effect estimates across traits, suggesting that pleiotropy within this pathway is largely regulated at the expression level. Unlike other traits studied in the US-NAM population to date such as flowering time, plant height, and inflorescence architecture, many significant pairwise epistatic interactions were detected between carotenoid QTL, further elucidating regulatory dynamics of the precursor and core pathways. The extensive information provided by these analyses regarding networks of additive, epistatic and pleiotropic QTL effects and their magnitudes can be readily incorporated into marker-based global biofortification breeding programs to achieve target nutritional profiles in maize grain.

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P350

New insights on *Cellulose Synthase-Like D1* controlling organ size in maize

(submitted by Weiya Li <liweiya@cau.edu.cn>)
Full Author List: Li, Weiya[1]; Chen, Qiuyue[1]; Yang, Zhixing[1]; Yao, Jieyuan[1]; Li, Jiansheng[1]; Tian, Feng[1]; Song, Weibin[1]; Yang, Xiaohong[1]
[1] National Maize Improvement Center of China, Beijing Key Laboratory of Crop Genetic Improvement, China Agricultural University, Beijing,100193,China

Genes involved in cell division regulation often affect plant organ size and, ultimately, plant architecture, such as leaf width, as well as most agronomic traits in maize. Here, we show that a quantitative trait locus for multiple agronomic traits related to plant organ size, *qLW10*, reflects allelic variation in *ZmCSLD1*, which encodes a cellulose synthase-like D protein. Loss of ZmCSLD1 function correlated with smaller organs as a result of decreased cell division. The high expression in rapidly growing regions, the subcellular localization in the Golgi, and the transcriptional responses caused by knockout of *ZmCSLD1* suggested that *ZmCSLD1* encodes a β-linked glucosyltransferase in the cell wall biosynthesis pathway and alters organ size by regulating the synthesis of early cell wall components, which, in turn, affect cell division. In addition, intragenic complementation was investigated for two *Zmcsld1* alleles with nonsynonymous SNPs in different functional domains, and the mechanism of this complementation was determined to most likely be through homodimeric interactions. These results provide new insights into the regulation of *ZmCSLD1* and extend our knowledge of the molecular mechanism underlying plant organ development

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P351

Novel alleles for Goss' Wilt resistance to maize

(submitted by Julian Cooper <julianscottcooper@gmail.com>)
Full Author List: Cooper, Julian[1]; Lopez-Zuniga, Luis[2]; Wisser, Randall[3]; Balint-Kurti, Peter[2]; Jamann, Tiffany[1]
[1] Department of Crop Sciences, University of Illinois at Urbana-Champaign, Urbana, IL, 61801
[2] Department of Plant Pathology, North Carolina State University, and United States Department of Agriculture–Agricultural Research Service Plant Science Research Unit, Raleigh, NC 27695
[3] Department of Plant & Soil Sciences, University of Delaware, Newark 19716

Since its discovery in 1969, Goss' wilt and blight, a vascular disease caused by the gram-positive bacteria *Clavibacter michiganensis* subsp. *nebraskensis* (Cmn), has emerged as one of the top four diseases of maize in the United States and Ontario, Canada (Mueller et al., 2016) . In 2015 an estimated 139.8 million bushels of maize were lost due to this pathogen (Mueller et al., 2016). No source of complete resistance has been described for Goss' wilt, and little is known about the genetics and mechanisms of host resistance to Cmn. Our research utilizes the 282-line Goodman maize diversity panel, which has been used extensively to map quantitative traits (Flint-Garcia et al., 2005). We have evaluated this population for Goss' wilt and will use genome-wide association mapping to identify candidate genes. In parallel, a population of disease resistance introgression lines (DRILs; Oh7B x NC344) has been evaluated. This population includes 265 unique lines with introgressions that tile the maize genome and will be used to map and characterize regions underlying Goss's wilt resistance. Preliminary findings have revealed a novel resistance QTL, and trials in 2017 will serve to confirm these findings. Future studies will also employ a GFP-transformed isolate of Cmn to delineate the underlying mechanism(s) by which differing QTL protect against vascular infection.

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P352

Phosphate nutrition during early growth of landrace maize (Zea mays L.) originating from the volcanic soils of the mexican highlands

(submitted by Michael Anokye <anomic17@gmail.com>)
Full Author List: Anokye, Michael[1]; González, Eric[1]; Salazar-Vidal, Miriam Nancy[1]; Aguilar, Chio[1]; Sawers, Ruairidh[1]
[1] Langebio-Cinvestav, Mexico, Irapuato, Guanajuato, C.P. 36821

Phosphorus (P) is an essential macronutrient required for plant development and reproduction. As a consequence, P is one of the main components of the fertilizers required to sustain modern agriculture. The primary source of P for plants is inorganic phosphate (Pi) taken up from soil solution. While typically abundant, P is one of the least available nutrients to the plant, due to chemical fixation and the formation of organic complexes. Root hairs are sub-cellular outgrowths from root epidermis that play an important role in the uptake of immobile nutrients such as phosphorus by increasing the volume of soil explored by root. In P-limiting soils, early establishment of root hairs by the developing root system, which marks a transition from using stored seed P to acquired P, is critical for subsequent plant growth and development, requiring the young seedling to rapidly establish an effective means of P uptake. This project aims to evaluate the potential of Palomero Toluqueño maize landrace, originating from P-limiting acidic volcanic soils of central Mexico, as a source for improved phosphate uptake during early growth of maize. Using agar solidified culture system in a controlled environment, I will evaluate and map QTLs (under +/-P) linked to root hair length in a 100 recombinant inbred lines (RILs) derived from a cross between maize genotypes B73 and Palomero Toluqueño (PT), which have contrasting adaptation to low phosphorus availability in the field. I will assess the extent to which positive alleles originate from the PT parent, and investigate any interaction between QTL effect and P treatment. Together, using semi-quantitative RT-PCR analysis, I will assess root tissue expression of Pi transporters encoded by members of the Pht1 gene family reported in maize on the basis of prior genome analysis investigated under phosphorus depleted environment.

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P353

Photosynthesis and grain yield in the field environments managed for drought stress during flowering: QTL mapping for testcross performance in IBM population

(submitted by Vlatko Galic <vlatko.galic@poljinos.hr>)
Full Author List: Galic, Vlatko[1]; Franic, Mario[1]; Simic, Domagoj[1]
[1] Department of maize breeding and genetics, Agricultural Institute Osijek, Juzno predgrade 17, Osijek, Croatia, HR31103

Measurements of chlorophyll *a* fluorescence transient present a powerful tool for evaluation of plant vitality in stress environments through probing the light induced energy fluxes in photosynthetic apparatus. Identification of QTLs and genes underlying chlorophyll *a* fluorescence has been done using a variety of methods, but QTLs for the fast chlorophyll *a* fluorescence transient quantified by JIP-test parameters, as a reliable early indicator of drought stress in the field environments are yet to be identified in maize testcrosses. Our experiment was set in six environments in Croatia and Turkey: three rainfed and three water-managed (water withholding during flowering), respectively, for testcrosses of 191 intermated recombinant inbred lines of the IBM maize population previously genotyped with ~107 000 markers. A tester of Iodent background was used. Initially, QTL analysis was performed using Haley-Knott regression with one QTL per chromosome model for, RC/ABS (QA–reducing centers per PSII antenna chlorophyll), PI_{ABS} (performance index for energy conservation from photons absorbed by PSII to the reduction of intersystem electron acceptors) and grain yield. As expected for complex traits, a larger number of significant but environmentally dependent QTLs was detected for RC/ABS and PI_{ABS} (LOD scores >4.2, chromosomes 4, 5 and 9) and grain yield (LOD scores >3, chromosomes 1, 4, 6, 8 and 10). Our results indicate there is a complex genetic structure for the two presented JIP-test parameters in maize testcrosses grown in drought stressed field environments but there could be a pattern of QTL effects expressed as functions of different drought scenarios. Our forthcoming complete quantitative genetic analysis of a larger field data set for series of photosynthetic and agronomic traits could be used for assessing the real-time photosynthetic performance of maize genotypes and the contribution of genomic regions under stress situations as a function of grain yield in drought-prone environments.

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P354

Progress in the development of Quality Protein Popcorn (QPP)

(submitted by Ying Ren <renying900115@hotmail.com>)
Full Author List: Ren, Ying[1]; Rose, Devin[2]; Rodriguez, Oscar[1]; Gaussoin, Roch[1]; Holding, David[1]
[1] Department of Agronomy and Horticulture, University of Nebraska, Lincoln, NE
[2] Department of Food Science and Technology, University of Nebraska, Lincoln, NE

Quality Protein Maize (QPM; modified *opaque-2*) has nearly double the amount of two essential amino acids (Lys and Trp) and is considered as a more balanced source of nutrition for humans and monogastric animals compared to normal dent corn. Availability of in-gene *o2* SSR makers has facilitated the conversion of vitreous endosperm popcorn to QPM. We started introgression of the *o2* allele into popcorn by making F_1 crosses between several elite popcorn lines and QPM lines. After selfing with concurrent backcrossing of selected partially modified BC_2F_2 individuals (type II and type III opaques), we characterized *o2* mutants with fully modified kernels in BC_2F_3, which were further self-pollinated for selection. With BC_2F_5 generation advancement underway, we aim to: (1) maximize recovery of popcorn genome; (2) carry out amino acid profiling and popping analysis; (3) assess agronomic traits of QPM conversion lines compared to popcorn lines. Recent progress and plans in this project will be presented.

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P355

QTL Analysis of Cold Tolerance in Maize

(submitted by Raeann Goering <rgoering01@hamline.edu>)
Full Author List: Goering, Raeann N[1]; Larsen, Siri[1]; Tan, Jia[1]; Whelan, James[1]; Makarevitch, Irina[1]
[1] Hamline University;1536 Hewitt Ave St Paul, MN, 55104

With booming populations soon to overwhelm the world's food production capabilities, studying what makes crop organisms, like maize, efficient is crucial to ensure that the demand for food is met. Planting earlier in the spring would lengthen the crop season and produce larger yields provided the crop is tolerant to early spring's low temperatures. In this study, a quantitative trait locus analysis was conducted using 97 lines of the IBM population and their parents (B73 and Mo17) in hopes of elucidating the differences in cold response between cold susceptible B73 and cold tolerant Mo17 maize lines. Cold tolerance phenotypes of chlorophyll concentration, percent green calculated from an image analysis of leaves and a qualitative three point scale were used to quantify response after plants had been stressed at 4℃ for 8 hours. Four QTL regions on chromosomes 1, 3, 5 and 7 were well supported with combinations of our phenotype data. Differential expression data from previous cold stress experiments were used to identify significantly differentially expressed genes within the QTL regions. Exploration of genetic variation found between B73 and Mo17 in these candidate genes revealed 14 genes that contain natural variation between the lines. These genes potentially explain the difference in cold stress response between B73 and Mo17 and could be selected for to breed cold tolerance in maize.

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P356

QTL mapping for days to antithesis (DTA) and days to silk (DTS) in multiple maize-teosinte hybrid populations

(submitted by Lora Daskalska <ldaskalska@wisc.edu>)
Full Author List: Daskalska, Lora L[1]; York, Alessandra M[1]; Yang, Chin Jian[1]; Doebley, John F[1]
[1] Department of Genetics, University of Wisconsin, Madison, Wisconsin, United States

Quantitative traits are controlled by multiple genes in the genome, and much are still unidentified today. To find causal quantitative trait loci (QTL) or genes, scientists have employed QTL mapping studies. The genes responsible for the domestication of maize (Zea mays L. ssp. mays) from teosinte [Z. mays ssp. parviglumis] have been identified by this type of approach (Clark et al. 2006, Wang et al. 2005, Wills et al. 2013). Because we find that different mapping population structures result in unique QTL, we have created a study with four teosinte-maize hybrid (BC1S4) populations that were developed using four separate teosinte inbred lines (TIL01, TIL03, TIL11, TIL14) and backcrossed to W22. Many phenotypes were scored in the four populations including days to antithesis (DTA) and days to silk (DTS). QTL mapping was performed on each population using R/qtl. Three to twelve QTL were found in each population, with each controlling 3-32% of the phenotypic variance. The QTL of largest effect for both DTA and DTS, which maps to chromosome 10, was responsible for the most percent variance explained; an average of 18% per population. Furthermore, the average Single Plant and Plot Basis Heritability for DTA and DTS was high at 88%. Our results appear to be consistent with previous results (Buckler et al. 2009) that multiple QTL across the genome appear to be significant for controlling these two traits.

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P357

QTL Mapping for *Fusarium* ear rot resistance in the MAGIC maize population

(submitted by Popi Septiani <popi.septiani@santannapisa.it>)
Full Author List: Septiani, Popi[1]; Lanubile, Alessandra[2]; Busconi, Matteo[2]; Inzé, Dirk[3]; Morgante, Michele[4]; Pè, Mario Enrico[1]; Dell'Acqua, Matteo[1]; Marocco, Adriano[2]
[1] Institute of Life Sciences Scuola Superiore Sant'Anna; Pisa, Italy, 56127
[2] Istituto di Agronomia, Genetica e Coltivazioni erbacee, Università Cattolica del Sacro Cuore; Piacenza, Italy, 29122
[3] Vlaams Instituutvoor Biotechnologie; Ghent, Belgium, B-9052
[4] Institute of Applied Genomics; Udine, Italy, 33100

Fusarium ear rot (FER), caused by *Fusarium verticillioides*, is a major threat to maize yield and grain quality worldwide. Genomic loci responsible for natural disease resistance can be identified through quantitative trait loci (QTL) mapping that allows the development of resistant lines for the sustainable control of FER. A Multi-parent Advance Generation Intercross (MAGIC) population in maize was recently developed, providing a mean to conduct high-definition QTL mapping on small sets of highly diverse recombinant lines. We performed an *in vitro* assay for FER resistance using a rolled towel method in a set of 400 MAGIC maize recombinant inbred lines (RIL). For each RIL, 20 kernels as control and 20 kernels inoculated with *F. verticillioides* conidia were germinated for seven days. We measured infection severity level (SEV), seedling weight (PW), and length (PL). QTL analysis was performed on RIL whose haplotypes were reconstructed from 50K single-nucleotide polymorphism data. We identified 11 high confidence QTL for the considered traits. We found two QTL on chromosome 4 and one QTL on chromosome 5 for SEV, confirming that FER resistance is controlled by multiple loci with low effect. To guide the identification of candidate genes within the identified QTL, we exploited transcriptomic and sequencing information generated on the founder lines. We tested the differential expression of genes in the QTL confidence intervals matching the founder effects at the QTL as estimated by the mapping model. We identified 32 suggestive candidates for the three traits. Finally, we imputed the full genome sequence of the founder lines on the reconstructed RIL haplotypes to perform GWAS in the QTL regions and narrow down the confidence intervals. We conclude that the rolled towel assay applied on the MAGIC maize population is a fast and cost-effective method to identify QTL and candidate genes for disease resistance in maize.

P358

QTL mapping for tiller number in multiple maize-teosinte hybrid populations

(submitted by Craig DeValk <cdevalk@wisc.edu>)
Full Author List: DeValk, Craig[1]; York, Alessandra[1]; Doebley, John[1]
[1] University of Wisconsin Madison, Madison, WI, USA 53706

Maize (*Zea mays* ssp. *mays*) and teosinte (*Zea mays* ssp. *parviglumis*) differ in many morphological and physiological characteristics. Quantitative trait locus (QTL) mapping studies have been regularly conducted to elucidate these differences, which were the result of domestication, and determine the underlying genetic architecture as well as find any causative genes. Many important domestication genes have been found this way including ones for ear diameter, kernel row number, and grassy tiller. Different mapping populations result in different QTL. In this study four teosinte-maize hybrid (BC_1S_4) populations were developed using four separate teosinte inbred lines (TIL01, TIL03, TIL11, TIL14) and backcrossed to W22. Multiple phenotypes were scored, including tiller number and performed QTL mapping on each population separately using R/qtl. Multiple significant QTL were found, with LOD scores ranging from 3.82 to 19.3 and percent variance explained ranging from 3.6-17.7%. There did not appear to be any previously known genes or transcription factors in the regions near any of the known QTL. In all populations, the QTL were found across multiple chromosomes indicating the tiller number trait is likely to be explained by a variety of genes and transcription factors.

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P359

Quantitative analysis of the maize leaf microbiome

(submitted by Jason Wallace <jason.wallace@uga.edu>)
Full Author List: Wallace, Jason G[1]; Moghadam, Mohsen[1]; Voghoei, Sahar[2]; Kremling, Karl A.[3]; Chen, Shu-Yun[4]; Su, Mei-Hsui[4]; Pardo, Jeremy D.[4]; Budka, Joshua S.[5]; Lepak, Nicholas K.[5]; Buckler, Edward S[3 4 5]
[1] Department of Crop and Soil Sciences, The University of Georgia, Athens, GA
[2] Department of Computer Science, The University of Georgia, Athens, GA
[3] Department of Plant Breeding and Genetics, Cornell University, Ithaca, NY
[4] Institute for Genomic Diversity, Cornell University, Ithaca, NY
[5] United States Department of Agriculture – Agricultural Research Service, Ithaca, NY

Maize plants do not grow in a vacuum but live in association with trillions of microscopic organisms that grow around, on, and inside them. Collectively, these organisms are referred to as the maize microbiome. We know very little about how these organisms interact with plants in the field, yet they could be an important way to improve 21st-century agriculture. To help understand how the genetics of maize plants influences their microbiome communities, we analyzed the bacterial leaf microbiome across ~270 diverse maize inbred lines grown in a single field. The bacterial community on maize leaves is very low diversity, with 20-30 common bacterial species dominating the majority of samples. The relative abundance of these species can vary dramatically by sample, and heritability analysis of this variability (that is, treating species abundance as a quantitative trait) indicates only a few species show significant influence from host genetics. We also show significant within-plot variation of the leaf microbiome, indicating that environmental noise plays a large part in community assembly. This noise could be because the plant genetics do not influence these communities strongly, because patchy microbial distributions lead to large sampling variability, or some combination of the two (possibly with other factors also involved). We indicate ways that these influences could be detangled, and we show preliminary data that microbes in other compartments (especially those that grow inside plant tissues) may be under much larger genetic influence from the host plant.

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P360

Relationship between maize (*Zea mays* L.) whole plant silage quality and ear morphological characteristics per whole plot basis

(submitted by Jonathan Renk <jrenk@wisc.edu>)
Full Author List: Renk, Jonathan[1]; Kaeppler, Shawn[1]; de Leon, Natalia[1]
[1] Department of Agronomy, University of Wisconsin-Madison, Madison, WI, USA 53706

Historically, improvements in maize forage yield and quality have been closely linked to improvements in grain yield productivity (Lauer et al., 2001). The goal of this study was to investigate the relationship between silage quality on a whole plot basis and ear morphological characteristics such as ear length, ear width, cob length, cob width, kernel row number, and plot weight for 96 maize recombinant inbred lines (RILs) derived from three different populations [32 intermated B73 x Mo17 (IBM), 32 Oh43 x W64A (OhW), and 32 Ny821 x H99 (NyH)] evaluated as testcrosses using inbred PHG47 as the tester. The RIL testcrosses were selected because they represented phenotypic extremes for forage biomass yield and digestibility. Hybrids were evaluated in replicated field trials across two environments in 2016 in South Central Wisconsin. Broad-sense heritabilities for ear traits ranged from 0.73 to 0.88 for IBM, 0.48 to 0.88 for NyH, and 0.24 to 0.86 for OhW. Significant differences occurred between extreme categories for OhW in kernel row number and cob width. Positive correlations were identified between ear traits for all three populations. Investigating the relationships between silage quality and ear morphological traits could help in the development of superior maize silage varieties.

Source: Lauer, J. G., Coors, J. G., and Flannery, P. J. 2001. Forage yield and quality of corn cultivars developed in different eras. Crop Science, 41(5), 1449-1455.

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P361

Simulation results suggest stepwise selection algorithm picks up epistatic signals in the US maize nested association mapping panel

(submitted by Angela Chen <chen398@illinois.edu>)
Full Author List: Chen, Angela[1]; Lipka, Alexander[2]
[1] Department of Statistics, University of Illinois, Champaign, Illinois, 61820
[2] Department of Crop Science, University of Illinois, Urbana, Illinois, 61801

The plant genome-wide association study (GWAS) has been successful in identifying genomic loci with moderate- to strong associations with agronomically important traits. However, there are two common setbacks among the most frequently used statistical models in a GWAS: (i) they test only one marker at a time and (ii) they only consider additive effects. To address both of these drawbacks, we developed a stepwise epistatic model selection approach that quantifies the simultaneous contribution of multiple additive and pairwise epistatic loci towards trait variability. Using the TASSEL program as a foundation, we implemented a beta version of this approach. The resulting algorithms are available to the public free of charge, and further improvements to its computational efficiency are expected. To assess the statistical aspects of this approach, we conducted a simulation study in a subset of the US maize nested association mapping panel. A total of 1,900 traits that varied in heritability and genetic architecture were simulated. Our results suggest that the stepwise epistatic model selection approach is capable of correctly identifying epistatic genomic signals and that these signals are rarely misclassified as additive.

P362

Small ad hoc versus large general training populations for genomewide selection in maize biparental crosses

(submitted by Sofia P. Brandariz <bran0795@umn.edu>)
Full Author List: Brandariz, Sofia P.[1]; Bernardo, Rex[1]
[1] Dep. of Agronomy and Plant Genetics, Univ. of Minnesota, 411 Borlaug Hall, 1991 Upper Buford Circle, Saint Paul, MN 55108

Historical phenotypic and marker data in a maize (*Zea mays* L.) breeding program can be used to develop a single, large training population for genomewide selection within each of several A/B biparental crosses. An alternative approach is to create separate but smaller training populations that maximize the relatedness between the test and training populations. Our objective was to determine if predictive ability and selection response in an A/B cross are higher with a single but very large training population or with ad hoc but smaller training populations. A total of 969 biparental maize populations from Monsanto were evaluated at 4-12 environments in 2000-2008 for yield, moisture, and test weight. The parents of the 969 populations were genotyped with 2911 single nucleotide polymorphism (SNP) markers, and marker data were imputed from lower-density screening of the progeny in each biparental cross. Training populations were constructed as follows: (i) general combining ability (GCA) model, in which the training population for the A/B cross consisted of all prior populations with A as one parent (A/*, where * is a related inbred) and with B as one parent (*/B); (ii) A/* populations only or */B populations only; and (iii) all */* crosses. Predictive ability and selection response were highest with the GCA model. Including only the A/* populations or only the */B populations generally reduced the predictive ability and selection response. Even though the single general training population (*/*) was 6 to 58 times larger than the GCA-model training population, such larger training population did not lead to a higher predictive ability or selection response. Filtering the */* populations to increase similarity with the A/B population was ineffective for increasing predictive ability and selection response. We concluded that it is best to design an ad hoc training population for each A/B biparental population.

Funding acknowledgement: Monsanto

P363

Stability and tradeoff of allelic effects in drought-treated elite temperate and tropical maize hybrids

(submitted by Addie Thompson <thomp464@purdue.edu>)
Full Author List: Thompson, Addie M[1]; Tuinstra, Mitchell R[1]
[1] Purdue University; West Lafayette, IN, USA 47907

Drought tolerance is becoming an increasingly high-value trait in maize. Due to the difficulty of performing controlled drought field trials, our current understanding the genetics of drought responses is limited. This is particularly true in the context of testcross hybrids representing the diversity of elite temperate and tropical germplasm. Here, recently expired Plant Variety Protection (ex-PVP) inbred lines and Drought Tolerant Maize for Africa (DTMA) inbred lines were crossed to two common testers, PHP02 and 2FACC, and grown over two or three years, respectively, in controlled-irrigation drought trials in Arizona. Crop adaptation phenotypes (flowering time, plant and ear height, stay-green, yield, and test weight) were collected under well-watered and drought conditions. Traits were linked to genetic variation via a Genome-Wide Association Study (GWAS) to investigate the basis of drought-affected phenotypes. Estimated marker effects were compared between populations, treatments, and testers to look for stability and tradeoff for individual alleles or haplotypes. Characterization of temperate and tropical germplasm using genome-wide marker effects revealed untapped genetic potential and target haplotype regions for improving drought tolerance in maize.

Funding acknowledgement: Howard Buffett Foundation

P364

Stomatal behavior and transcriptional response of 27 maize genotypes to drought

(submitted by Jeremy Pardo <jdp267@cornell.edu>)
Full Author List: Pardo, Jeremy D.[1 2]; Kremling, Karl A.[1]; Lepak, Nicholas K.[3]; Bauerle, Taryn L.[2]; Buckler, Edward S.[1 3]
[1] Section of Plant Breeding and Genetics, School of Integrative Plant Science, Cornell University; 175 Biotechnology Building Ithaca NY 14853
[2] Section of Horticulture, School of Integrative Plant Science, Cornell University; Plant Science Building, Ithaca, NY 14853
[3] USDA Robert Holley Center; Ithaca, NY 14853

Optimal stomatal conductance under drought is a balance between maximizing CO2 assimilation necessary for photosynthesis, and prevention of water loss. The transcriptional control of stomatal closure is critical in regulating this balance. To further our understanding of differential stomatal behavior and transcriptional response to drought, we conducted an experiment where we withheld water from three-week old seedlings of the 27 maize Nested Association Mapping (NAM) founder lines in a greenhouse each genotype, for three days following drought stress initiation. We identified significant differences in stomatal conductance of over 100 under μmol M-2 S-1 drought among the NAM founders. To detect genes correlated with stomatal behavior during drought, we constructed 3' RNAseq libraries from leaf tissue collected one and three days after drought initiation as well as from well-watered controls on each day. We identified ~650 genes differentially expressed between control and drought conditions after one day of drought stress and ~7000 genes differentially expressed after three days of drought stress. Gene co-expression network analysis was conducted using a combination of K-means and hierarchical clustering to identify modules of co-expressed genes based on their expression pattern under drought. We identified 44 total modules, four of which had expression patterns that were significantly correlated with stomatal conductance under drought. Gene ontology enrichment analysis revealed significant enrichment for stomatal movement genes in one of these modules. Future studies will examine transcription factors associated with modules correlated with stomatal conductance, to generate hypotheses about the regulators of expression for drought-induced stomatal closure genes.

Funding acknowledgement: United States Department of Agriculture (USDA), American Society of Plant Biologists (ASPB)

P365

The challenge of poor penetrance by *pangloss1* while breeding maize lines to test its impacts on yield

(submitted by Madeline McMullen <mmcm@iastate.edu>)
Full Author List: McMullen, Madeline L.[1]; Doblas Ibanez, Paula A.[2]; Posekany, Tes[1 3]; Smith, Laurie G.[2]; Lauter, Nick[1 3 4]
[1] Department of Plant Pathology and Microbiology, ISU, Ames, IA 50011
[2] Department of Cell and Developmental Biology, UCSD, La Jolla, CA 92093
[3] Interdisciplinary Genetics and Genomics Graduate Program, ISU, Ames, IA, 50011
[4] USDA-ARS Corn Insects and Crop Genetics Research Unit, Ames, IA, 50011

PANGLOSS1 is a leucine-rich repeat receptor-like kinase protein that promotes polarization during asymmetric cell divisions required for normal stomate development in Maize (Cartwright et al., 2009; Science v323, p649-651). The *pan1-ems* allele acts recessively to cause abnormal morphology of stomatal subsidiary cells. Microscopic examination of super glue impressions of the leaf epidermis show that the *pan1-ems* phenotype is quite obvious and fully penetrant in a B73 genetic background. Separately, a role for the *pan1* gene in disease defense was uncovered by Jamann et al., 2014; (Genetics, v198, p333–344), who identified it as a candidate gene at a multiple disease resistance locus. Jamann and colleagues demonstrated that *pan1* gene expression was lower in the QTL allele that conferred resistance to both *Setosphaeria turcica* and *Pantoea stewartii*, the causal agents of Northern Leaf Blight and Stewart's Wilt, respectively. They also showed that *pan1-ems/pan1-ems* plants were more resistant than wildtype plants, confirming the viability of the candidate gene hypothesis. Strikingly, Jamann and colleagues also reported preliminary results indicating that *pan1-ems*/+ plants were more resistant to these diseases than wildtype plants. Thus, a paradox in the mode of action for *pan1-ems* exists: in stomate morphology it acts recessively, but in disease defense it acts dominantly. To investigate this paradox in multiple environments and in both inbred and hybrid genetic contexts, backcross breeding into Mo17 and B73 was conducted. During this process, poor penetrance by *pan1-ems* in the Mo17 background was uncovered. Here we report the development and use of molecular markers and progeny testing to assure proper dosages of the *pan1-ems* allele in these experiments. We will also discuss the experimental design for yield testing with and without disease pressures.

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

P366

The disease profile of the brown midrib mutants in maize

(submitted by Judith Kolkman <jmkolkman@gmail.com>)
Full Author List: Kolkman, Judith M[1]; Repka, Anne[1]; Swarts, Kelly[3]; Balint-Kurti, Peter[2]; Nelson, Rebecca J.[1]
[1] School of Integrated Plant Sciences, Cornell University, Ithaca, NY USA 14853
[2] U.S. Dept. of Agriculture, USDA-ARS, Plant Science Research Unit, Raleigh, NC USA
[3] Max Planck Institute for Developmental Biology , Tubingen, Germany

Recent epidemics of northern leaf blight (NLB) on brown midrib (BMR) silage corn hybrids led to the hypothesis that the BMR alleles may enhance vulnerability to fungal diseases. BMR lines have mutations in genes that influence lignin content, which is desirable in silage corn because of its greater digestibility for ruminants. These genes are part of the phenylpropanoid pathway, which plays a role not only in structural support, but also plant defense. To date, six brown midrib mutants have been described in maize, with four of them defined to the gene level. In this study, we investigate the role of the brown midrib genes as candidate genes for resistance to multiple diseases in maize through disease phenotyping and genic association analysis. Several brown midrib genes are near to or within QTL intervals for NLB. Field inoculation of *bm1*, *bm2*, *bm3* and *bm4* mutant lines with the NLB, Stewart's wilt and grey leaf spot pathogens indicated that the mutants are highly susceptible to all three foliar diseases. Natural genetic variation at *bm2* was associated with NLB and GLS resistance in the 282-line maize diversity panel, allelic variation at *bm1* was associated with southern leaf blight. Understanding the disease vulnerabilities of BMR maize is important for resistance breeding and complementary disease management strategies.

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P367

The effect of artificial selection on phenotypic plasticity in maize

(submitted by Joseph Gage <jgage2@wisc.edu>)
Full Author List: Gage, Joseph[1]; Jarquin, Diego[2]; Romay, Cinta[3]; Lorenz, Aaron[4]; Buckler, Edward S.[5]; Kaeppler, Shawn[1]; GxE Consortium, [6]; de Leon, Natalia[1]
[1] Department of Agronomy, University of Wisconsin-Madison, 1575 Linden Drive, Madison, WI, U.S.A. 53706
[2] Department of Agronomy and Horticulture, University of Nebraska-Lincoln, 321 Keim Hall, Lincoln, NE, U.S.A. 68583
[3] Institute for Genomic Diversity, Cornell University, 175 Biotechnology Building, Ithaca, NY, U.S.A. 14853
[4] Department of Agronomy and Plant Genetics, University of Minnesota-St Paul, 1991 Upper Buford Circle, St Paul, MN, U.S.A. 55108
[5] USDA-ARS Plant, Soil, and Nutrition Research Unit; Institute for Genomic Diversity, 159 Biotechnology Building, Ithaca, NY, U.S.A. 14853
[6] http://www.genomes2fields.org/

Remarkable productivity levels have been achieved in crop species through artificial selection and adaptation to modern agronomic practices. Whether this intensive selection has also changed the ability of improved cultivars to maintain high productivity across variable environments is unknown. Deeper understanding of the genetic control of phenotypic plasticity and genotype by environment (G×E) interaction will enhance the ability to predict crop performance across diverse environments. We used the Genomes to Fields (G2F) G×E Maize project to assess the effect of selection on G×E variation and characterize polymorphisms associated with plasticity. Genomic regions displaying evidence of selection during modern temperate maize breeding were identified by evaluating Fst between pools of temperate and tropical inbred lines. Selected regions explained less variability for yield G×E than unselected regions, indicating that improvement due to breeding efforts may have reduced G×E of modern cultivars. A Finlay-Wilkinson regression was used to quantify and map hybrid stability. Loci associated with stability were evaluated for distance from the closest gene to identify trends in genomic position of variants controlling stability. We observed enrichment of variants 0-5,000 base pairs upstream of genes and a decrease in genic associations, hypothetically due to control of plasticity by short-range regulatory elements.

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P368

The FLOURY2 zein-RFP transgene – a phenotype to investigate multiple modes of regulation

(submitted by Nicholas Heller <njhelle2@illinois.edu>)
Full Author List: Heller, Nicholas J[1]; Tiskevich, Christine J[1]; Moose, Stephen P[1]
[1] University of Illinois; Urbana, Illinois, USA, 61801

The genetic control of zeins is not well understood because the zeins are a large gene family with complex inheritance and a strong influence from environmental factors, such as soil nitrogen supply. The Floury2 zein-RFP (Red Fluorescent Protein) fusion protein is an excellent reporter gene to simplify some of these issues because it is tracking a single alpha-zein, it can be visualized non-destructively, and can be quantitatively measured through imaging techniques. We've used this tool to explore the effect of genotype, hybridization, epigenetic variation, and response to nitrogen fertilizer. The Illinois Long Term Selection Experiment (ILTSE) has produced the known phenotypic extremes for grain protein, known as Illinois High Protein (IHP) and Illinois Low Protein (ILP). Much of this response to selection was in the zein proteins, and the RFP reporter effectively tracks the full range of values and behaves as zeins do even in such extreme genomes. To address the question of inheritance following hybridization, hybrids were made with the Illinois Protein Strains Recombinant Inbred (IPSRI) Lines. Several agronomic traits were measured on the hybrids in addition to the zein-RFP reporter phenotype which revealed important relationships between the RFP phenotype and agronomic traits. Additionally, there is a proposed epigenetic component to the regulation of zeins. The dramatic response of the ILTSE is difficult to imagine in the context of allelic variation as all the ears by cycle 10 could be traced back to one original ear. Here, a population of epiNILs in the B73 inbred background, potentially exhibiting epigenetic variation, was shown to yield a wide variation in the RFP reporter when there is very little variation in the control. Finally, the RFP reporter also shows a strong response to the environmental application of nitrogen fertilizer.

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P369

The genome-wide association study dissects the genetic architecture of embryo size in maize

(submitted by Xiaowei Li <lixiaowei810@126.com>)
Full Author List: Li, Xiaowei[1]; Li, Jiansheng[1]; Yang, Xiaohong[1]
[1] National Maize Improvement Center of China, China Agricultural University, Beijing 100193, China

Mature embryo accounts for about 10% of maize kernel dry weight, and accumulates nearly 80% of kernel oil and considerable lipid-soluble vitamins, playing a critical role in controlling the balance between grain nutrition and yield. However, the genetic basis of maize embryo size is poorly understood. In this study, more than 30,000 embryos from three environments were assayed in a natural population of 513 inbred lines and 49 embryo size associated loci were identified by genome-wide association study (GWAS) using mixed linear model at $P < 1.8 \times 10^{-6}$. Among these 49 loci, 23 showed pleiotropism and 39 were supported by eQTLs. The largest contributor to the variation of embryo mass was embryo width, whereas the embryo length and thickness showed moderate variation. In addition, phenotypic analysis and GWAS indicated that embryo size and kernel size varied in an uncoordinated manner. Furthermore, the associations between two key candidate genes and embryo size were validated by *Mu* mutations and the CRISPR/Cas9 based knockout system. These results will provide insights into the genetic basis of variation of embryo size in maize.

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P370

The high-amylose trait of GEMS-0067 is due to an allele of *starch branching enzyme 1* that appears to be literally wild-type

(submitted by Donald Auger <donald.auger@sdstate.edu>)
Full Author List: Auger, Donald[1]; Gyawali, Abiskar[2]; Wu, Yusheng[3]; Huang, Ruijia[1]; Wu, Yajun[1]
[1] Dept. of Biology-Microbiology, South Dakota State University, Brookings, SD 57007, USA
[2] Div. of Biological Sciences, University of Missouri, Columbia, MO 65211, USA
[3] School of Arts and Sciences. University of the Southwest, Hobbs, NM 88240, USA

Starch in maize endosperm is predominantly amylopectin, which is a polymer of glucose that is highly branched. There are food and industrial applications that call for the relatively unbranched starch, *i.e.*, amylose. Maize kernels that are homozygous for recessive *amylose extender 1* (*ae1*) have endosperm starch that is about 50% amylose. One variety of maize, GEMS-0067, is homozygous *ae1*, but produces kernels that are singularly high in amylose, typically around 70%. We detected a strong quantitative trait locus (QTL) for the high amylose trait in an F2 population from a cross between GEMS-0067 and H99ae. This QTL, which accounted for most of the heritability of this trait, is additive and located on the short arm of chromosome 5 (5S). Near the peak of this QTL is *starch branching enzyme 1* (*sbe1*), which makes it a potential candidate. To test whether the allelic segregation of *sbe1* or, instead, some closely linked gene is responsible for the QTL, we introduced a null allele of *sbe1* that acts as a simple recessive. As expected, the progeny carrying the *sbe1* allele from GEMS-0067 have a higher proportion of amylose than those from H99ae, but neither of these two alleles segregating with the null *sbe1* allele display additivity of the trait. This directly implicates *sbe1* and justifies the analysis of the alleles. We sequenced the *sbe1* alleles from GEMS-0067 and 16 other divergent varieties. Comparisons indicate that *sbe1* from GEMS-0067 may be ancestral, showing close homology with two teosintes.

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P371

The hunt for modifiers of the *Tcb-1* locus

(submitted by Merritt Burch <merritt.b.burch@sdstate.edu>)
Full Author List: Burch, Merritt B[1]; Auger, Donald[1]
[1] South Dakota State University; Departmet of Biology and Microbiology; Brookings; South Dakota; 57006

Teosinte crossing barrier 1 (*Tcb1*) is a genetic cross-incompatibility system responsible for blocking non-self-type pollen in silks. Traditionally found in teosintes, *Tcb1-s* (strong allele) has been introduced into modern maize varieties conferring resistance to *tcb1* pollen. A previous quantitative trait loci (QTL) study of a similar cross incompatibility system, *Gametophye factor 1* (*Ga1-s*) has demonstrated that multiple modifying loci contribute to the effectiveness of silks at resisting foreign pollen types. Little is known about the genetic modifiers of *Tcb1* and, most importantly, what the underlying biological mechanism is for this cross incompatibility. An opportunity to investigate this was suggested by Jerry Kermicle, who observed that nearly all the F1's of various inbreds, including B73, crossed by W22 *Tcb1-s* demonstrate strong incompatibility with *tcb1* pollen. The one exception was Mo17, whose F1s had much weaker resistance. In our poster we will outline our study using the intermated B73 and Mo17 (IBM) population crossed with homozygous W22 *Tcb1-s* plants to test the efficiency of the various F1s at rejecting *tcb1* pollen. We produced the F1s this last summer and this next season we will test the efficacy of each of the F1 lines. To do that we will first test the *Tcb1* silks with colored R1-scm2 *tcb1* pollen and the next day pollinate the same silks with colorless *Tcb1-s* pollen. Mature ears will be scored for the proportion of colored kernels and the generated data will allow for QTL mapping of *Tcb1* modifying factors. Further knowledge of cross-incompatibility can be beneficial to breeders and farmers when only certain pollen types are desired on specialty maize crops.

Funding acknowledgement: SDSU Experimental Station

P372

The influence of peak GWAS signals on genomic prediction accuracy

(submitted by Brian Rice <brice6@illinois.edu>)
Full Author List: Rice, Brian R.[1]; Lipka, Alexander E.[1]
[1] 1University of Illinois Department of Crop Science, Turner Hall, MC-046 1102 South Goodwin Avenue Urbana, IL 61801

Some of the most important agronomic traits of interest are complex and thus governed by many genes of small effect. The genome-wide association study (GWAS) and genomic selection (GS) both use statistical models that quantify the contributions of these genes to trait variation. In general, the GWAS has been successful at identifying genomic regions containing markers with moderate to strong marker-trait associations. It is possible to incorporate markers tagging such peak GWAS signals into breeding programs through marker-assisted selection, where plants with favorable alleles at the peak GWAS signals are selected. In the absence of such signals, GS is typically effective at accurately predicting trait values. These two strategies have been used separately until recently, when the predictive ability of GS models that include peak associated markers from GWAS as fixed effect covariates was assessed. Theoretically, these models should be optimal for predicting traits that have several genes of large effect and many genes of smaller effect. We expand upon this work by evaluating simulated and real data from diversity panels in maize and other agronomically important crop species. Upon completion of this work, we anticipate being able to rigorously quantify the ability of fixed effect covariates tagging peak GWAS signals to increase GS prediction accuracy under a wide variety of genetic architectures and genomic backgrounds.

P373

Understanding plant functional morphology, water use and cellular composition using structured genetic populations of Setaria viridis

(submitted by Max Feldman <mfeldman@danforthcenter.org>)
Full Author List: Feldman, Max[1]; Ziegler, Greg[1]; Jurkowski, Melissa[1]; Baxter, Ivan[1]
[1] Donald Danforth Plant Science Center, 975 N. Warson Road, St. Louis, MO, 63132

Setaria viridis is proving to be an ideal experimental model to study the genetic the basis of ecophysiological traits in controlled environments. In addition to many favorable experimental and life history attributes, *S. viridis* exhibits high genetic diversity and has evolved the ability to colonize habitats throughout the globe. Understanding how the developmental processes that influence plant structure interact with physiological characteristics such as water use and nutrient assimilation is an important area of research. To achieve this objective we have performed three water limitation experiments using two independent genetically structured populations of *S. viridis* (A *S. viridis* natural diversity panel and an interspecific *S. italica* x *S. viridis* recombinant inbred line population) in the Bellweather Phenotyping Facility at the Donald Danforth Plant Science Center. The capabilities afforded by broad spectrum elemental profiling, high-frequency temporal trait measurement and stringent control of environmental variables have enabled us to rapidly identify germplasm with unique properties and gain insight into how genetic loci interact with abiotic factors to determine plant phenotype.

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P374

Understanding the regions that control grain yield with Ames and NAM hybrids

(submitted by Cinta Romay <mcr72@cornell.edu>)
Full Author List: Romay, Cinta[1]; Larsson, Sara[2]; Bradbury, Peter[3]; Buckler, Edward[1 2 3]
[1] Institute for Genomic Diversity, Cornell University, Ithaca, NY, USA 14853
[2] Plant Breeding and Genetics Section, Cornell University, Ithaca, NY, USA 14853
[3] U.S. Department of Agriculture (USDA) - Agricultural Research Service (USDA-ARS)

It has been proposed that complementation of alleles is one of the main components of hybrid vigor, and thus of high yielding maize hybrids. Low levels of recombination in certain regions of the genome make it difficult to purge out genetic load and favour that crosses between inbred lines that are adapted to similar environments but genetically diverse produce higher yields than crosses between closely related lines. To test how the regions of the genome controlling differences in grain yield are related to recombination and other genomic features, we evaluated a set of mostly non-elite hybrids created by crossing a subset of the US National Maize Inbred Collection (Ames) and a subset of the US Nested Association mapping population (NAM) - those whose flowering-time ranges were similar to B73 – with PHZ51 and/or PHB47. Nearly 3,000 hybrids were evaluated in 10 environments across the US over three years, for a range of developmental traits, as well as yield. The inbred lines have been genotyped using GBS (Genotyping by Sequencing) and hybrid genotypes were inferred by combining tester and inbred information. We used a variance components analysis, genome wide association analysis with a mixed linear model approach (GWAS), and a Bayesian whole genome regression (Bayes C) to study the genetic architecture of yield in these two diverse populations. Additionally we compared it to two other complex traits, flowering time and plant height. Cross prediction of yield between the two populations is low (r= 0.22 using Ames to predict NAM), proportions of variance explained by each of the chromosomes are different, and the location of the main GWAS hits in NAM do not match those from Ames, overall showing that yield is controlled by many small effects mostly not shared between the different subpopulations. However, some common patterns can still be observed between them. Our results suggest that modern temperate breeding has been successful at targeting several important regions reducing grain yield, but some difficult regions with lower recombination than average still play a key role controlling this complex trait.

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P375

Using X-ray computed tomography to quantify Micro- and Macro-Morphology of maize and other plants

(submitted by Keith Duncan <kduncan@danforthcenter.org>)
Full Author List: Duncan, Keith E[1]; Jiang, Ni[1]; Topp, Christopher N[1]
[1] Donald Danforth Plant Science Center; St. Louis, MO, USA 63132

Non-destructive sampling and analysis in plant biology have long been sought after to enable substantive research into the quantitative genetics of agronomic traits. X-ray computed tomography (CT) has been used widely since the 1970s in medical and industrial applications, but its use in plant biology has been sporadic at best. The Topp Lab at the Donald Danforth Plant Science Center obtained an industrial scale X-ray CT instrument in August 2016 and we have been exploring a wide range of applications for non-destructive imaging in plant biology since then. The system is versatile: it is capable of scanning samples as large as 2' x 2' x 4' at resolutions of ~100-150um, or as small as a seed at ~5um resolution. We have generated hundreds of 3D volumes from scanning above-ground structures such as sorghum panicles, grape buds and rachis', maize kernels, stalks and excavated root crowns, and a variety of plant seedlings. These 3D volumes can be used directly to quantify a variety of internal and external plant structures; for example, we've examined a developmental time course of NAM founder crown root architecture from field-excavated samples. However, a major challenge lies in the in-situ analysis of plant subterranean structures. The Topp lab and collaborators are developing computational segmentation methods to separate 3D root volumes from the surrounding soil matrix. Work will be presented on the in-situ identification of nodules from soybean roots, cassava storage root formation, and maize root architecture. Our aim is to generate robust methods for plant X-ray analysis to enable the research community in new understandings of plant development and genetics for basic and applied outcomes.
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P376

Validation of Multiple Disease Resistance loci in Maze using families derived from segment substitution lines

(submitted by Lais Bastos Martins <lbastos@ncsu.edu>)
Full Author List: Lopez-Zuniga, Luis O.[1]; Bastos Martins, Lais[1]; Wisser, Randall[2]; Holland, James B.[1 3]; Balint-Kurti, Peter[3 4]
[1] Dept. of Crop and Soil Science - North Carolina State University, Box 7616 Raleigh, NC 27995
[2] Dept. of Plant and Soil Science - University of Delaware, Newark, DE 19716
[3] USDA-ARS, Plant Science Research Unit, Raleigh, NC 27695-7620
[4] Dept. of Plant Pathology - North Carolina State University, Box 7616 Raleigh, NC 27695

Southern leaf blight (SLB), northern leaf blight (NLB), and gray leaf spot (GLS) caused by *Cochliobolus heterostrophus*, *Setosphaeria turcica*, and *Cercospora zeae-maydis* and *Cercospora zeina* respectively, are among the most important corn diseases worldwide. Previously, moderately high and significantly positive genetic correlations between resistance levels to each of these diseases were identified in a population of 253 diverse maize inbred lines. In this project we identified loci underlying disease resistance in multiple disease resistant (MDR) lines by the creation of chromosome segment substitution line (CSSL) populations in multiple disease susceptible (MDS) backgrounds. Four MDR lines (NC304, NC344, Ki3 and NC262) were used as donor parents and two MDS lines (Oh7B, H100) were used as recurrent parents to produce eight BC_3 $F_{4:5}$ CSSL populations comprising 1,749 inbred lines in total. Each population was assessed for each disease in replicated trials in two environments. Moderate to high heritabilities on an entry mean basis were observed (0.32 to 0.83). Several lines in each population were significantly more resistant than the susceptible recurrent parental lines for each disease. For most populations and most disease combinations, significant correlations were observed between scores and between marker effects for each disease. The number of lines with significant resistance to more than one disease was significantly higher than would be expected by chance. We were able to detect quantitative trait loci for disease resistance for each disease: 36 for SLB; 16 for NLB; and 20 for GLS. Among these, 30 QTLs were associated with variation in resistance to a single disease, 17 to two diseases (SLB/NLB: 5; SLB/GLS: 9; NLB/GLS: 3), and four to all three diseases. During summer 2017, we will validate and fine map QTL associated with MDR using $F_{2:3}$ backcross families derived from the 10 of the lines showing highest MDR.

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P377

Verification of highland/lowland adaptation in maize landraces by reciprocal transplantation

(submitted by Garrett Janzen <gjanzen@iastate.edu>)
Full Author List: Janzen, Garrett M.[1]; Aguilar-Rangel, María Rocío[2]; Rellán-Álvarez, Rubén[2]; Sawers, Ruairidh J. H.[2]; Hufford, Matthew B.[1]
[1] Iowa State University; 2200 Osborn Drive; Ames, Iowa, USA 50010
[2] Langebio Cinvestav; Km 9.6 Libramiento Norte Carretera León; Irapuato, Guanajuato, Mexico 36821

Maize landrace adaptations to highland and lowland conditions are traits of interest to researchers and breeders. In order to verify the existence of highland and lowland adaptations in maize landraces, we have performed a reciprocal transplant experiment across an elevation gradient in Mexico. Included in this experiment are four regional populations (Mexican highland, Mexican lowland, South American highland, South American lowland) with 30 landraces sampled from each region (120 landraces total). Each lowland landrace is coupled with a highland landrace from a similar latitude (within one degree) to permit comparisons between highland and lowland landraces while taking into account phenotypic covariance due to latitude. The two common garden sites that comprise this study are in the Pacific coastal lowlands of Mexico (54 m) and the highlands of the Mexican Central Plateau (2852 m). Phenotypic data of fitness traits (as well as macrohair density and anthocyanin pigmentation, which are putatively highland-adaptive) have been collected. Plants from each landrace will also be genotyped via GBS. By comparing phenotypic and genotypic data, a mean QST vs. mean FST comparison will be used to estimate average levels of highland/lowland adaptation in the four regional populations. Lower fitness values are expected to be observed for locally adapted landrace individuals grown outside of their ecological niche relative to those grown within their niche. As both common garden field sites are in Mexico, Mexican landraces may show greater fitness and/or evidence of local adaptation than the South American landraces.

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P378

Water use efficiency in hybrid maize

(submitted by Mengqiao Han <mhan16@illinois.edu>)
Full Author List: Han, Mengqiao[1]; Studer, Anthony J[1]
[1] University of Illinois Urbana-Champaign; Urbana ,IL, 61801, USA

Water use for agriculture accounts for the majority of freshwater usage on the planet. Furthermore, drought stress is an increasing threat to crop yield due to global climate change. Transpiration is a major source of plant water loss and occurs when stomata are open for gas exchange. Direct measurements of corn water use are difficult in the field, but measurement of the isotopic ratio of carbon in the leaf could be a possible high-throughput method. During CO_2 uptake and photosynthesis plants discriminate against the heavier $^{13}CO_2$ relative to the more abundant $^{12}CO_2$. The ratio of the carbon isotopes, termed δ13C, is an integrated measurement that is responsive to water stress, and may indicate the water-use efficiency of different lines. Previous research suggest there is great variability in leaf δ13C for corn inbreds. Because commercially grown corn is almost exclusively hybrid, it is important to understand δ13C in hybrids. This project focuses on the combinability of inbred lines with extreme δ13C values. Lines made from reciprocal crosses will enable us to determine parental isotopic effects. Hybrid lines made from crossing inbreds with high and low δ13C were planted in three different locations during the summer of 2016. Isotopic data and plant morphological traits were collected and analyzed to find possible correlations and/or parent of origin effects. This experiment will enhance our understanding of δ13C in hybrids and its relationship to agronomic traits in a field environment with the long-term goal of improving the water use efficiency of maize hybrids.

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P379

A method for enrichment of maize stem cells and leaf primordia

(submitted by William Ricci <william.ricci@uga.edu>)
Full Author List: Ricci, William A[1]; Zhang, Xiaoyu[1]
[1] Department of Plant Biology, University of Georgia, Athens, GA 30602

Plant development is characterized by continuous organ formation and growth throughout the life cycle. This is primarily achieved via two small groups of self-renewing stem cells at the shoot and root apical meristems (SAM and RAM, respectively). In the SAM, stem cells are maintained in a central zone and daughter cells move laterally to peripheral zones where they differentiate into lateral organs, such as leaves. Despite the biological importance of plant stem cell maintenance and differentiation, the underlying mechanisms of stem cell self-maintenance and differentiation remain outstanding questions in plant biology. This gap in our understanding derives largely from the technical difficulty of performing chromatin analyses on the small number of cells in the SAM. To resolve this, we are using cell-type-specific fluorescent lines, coupled with fluorescence-activated cell sorting, to acquire homogenous samples of stem cells and cells of young leaf primordia.

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P380

A sequenced-indexed reverse genetics resource for maize

(submitted by Mithu Chatterjee <cmithu@waksman.rutgers.edu>)
Full Author List: Chatterjee, Mithu[1]; Li, Yubin[1]; Wang, Qinghua[1]; Xiong, Wenwei[2]; Wang, Harrison[1]; Huang, Jun[1]; He, Limei[1]; Segal, Gregorio[1]; Du, Charles[2]; Dooner, Hugo[1]
[1] Waksman Institute of Microbiology, Rutgers University, Piscataway, NJ 08854, USA
[2] Montclair State University, Montclair, NJ 07043, USA

The availability of a mutant line in which a single known gene has been disrupted gives biologists a powerful tool in understanding the action of that gene. Thus, sequenced-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 created by *Agrobacterium*-mediated transformation. We are using a cost-effective method that takes advantage of next-generation sequencing (NGS) technologies. Specifically, our goals for this project 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 and, thus, enables researchers to generate regional gene knock-out collections; (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, eighty-six launching platforms have been mapped to all 20 chromosome arms of the maize reference genome. Along, we have mapped more than 10,000 transposed *Ds** target sites to the reference B73 genome with the help of our 3-dimensional DNA pooling strategy. All the lines generated in this project are listed in our database http://acdsinsertions.org, and more than 7000 of them have been already sent to the Maize Genetics Stock Center for distribution.

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P381

Abiotic stress induced nucleosome occupancy profiling in maize

(submitted by Thelma Madzima <madzima@uw.edu>)
Full Author List: Madzima, Thelma[1]
[1] University of Washington Bothell, School of STEM, Division of Biological Sciences; Bothell, WA, USA 98011

As sessile organisms, plants must adapt rapidly to fluctuating and often extreme environmental (abiotic) conditions. Transcriptional responses to abiotic stresses are also dependent on alterations to chromatin structure; including changes in nucleosome positioning, occupancy and composition. Chromatin structure is determined by several factors including DNA sequence, epigenetic modifications and the activity of ATP-dependent chromatin remodeling complexes. Understanding the mechanisms of abiotic stress-mediated chromatin remodeling in maize has far reaching effects on agricultural productivity; however, the association between these regulatory mechanisms remains largely uncharacterized. Herein, we profile chromatin structure to characterize genome-wide abiotic stress-induced changes in nucleosome occupancy in maize using a Differential (micrococcal) Nuclease Sensitivity and sequencing (DNS-seq) technique. This research will give insights into the mechanism of chromatin-remodeling in response to abiotic stress in maize.

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P382

Application of ATAC-seq to monitor variation in open chromatin among maize tissues and genotypes

(submitted by Jaclyn Noshay <nosha003@umn.edu>)
Full Author List: Noshay, Jaclyn M[1]; Lu, Zefu[3]; Hirsch, Candice N[2]; Schmitz, Robert J[3]; Springer, Nathan M[1]
[1] Department of Plant and Microbial Biology, University of Minnesota, St. Paul, MN
[2] Department of Agronomy and Plant Genetics , University of Minnesota, St. Paul, MN
[3] Department of Genetics, University of Georgia, Athens, GA

Chromatin accessibility is important for proper regulation of gene expression and can help to identify functionally relevant portions of the maize genome. The Assay for Transposase Accessible Chromatin sequencing (ATAC-seq) approach was successfully applied to several tissues of three inbred lines of maize (B73, PH207, and W22). This provided estimates of chromatin accessibility through the maize genome and identified numerous Accessible Chromatin Regions (ACRs). Many of these ACRs are found in the regions immediately upstream or downstream of maize genes. A subset of ACRs are found in intergenic regions and may represent distal regulatory elements. The ACRs all exhibit substantially reduced levels of CG and CHG methylation compared to flanking regions. Comparisons of ACRs among different genotypes provides an opportunity to assess the conservation of ACRs among haplotypes and to assess the impact of large insertions or deletions on the location of ACRs relative to genes. RNAseq was performed in the same tissues to allow for a direct comparison of chromatin accessibility and gene expression. The variation in gene expression among either different tissues or different genotypes was compared with variation in accessible chromatin to understand the relationship between changes in gene expression and open chromatin. Comparisons of ACRs near genes with tissue-specific expression or genotype-specific expression may reveal the factors that contribute to differences in chromatin accessibility. These analyses of chromatin accessibility in maize can help to identify important regulatory regions and can contribute to our understanding of functional variation in the maize genome.

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P383

Cell-autonomous action of RNA polymerase IV maintains the epigenetic repression of a paramutant *pl1* allele

(submitted by Allison McClish <mcclish.23@osu.edu>)
Full Author List: McClish, Allison[1]; Giacopelli, Brian[2]; Hollick, Jay B.[1 3]
[1] Department of Molecular Genetics, The Ohio State University
[2] Molecular, Cellular and Developmental Biology Graduate Program, The Ohio State University
[3] Center for RNA Biology, The Ohio State University

Paramutation describes a heritable epigenetic change in gene regulation initiated by trans-homolog interactions[1] and maintained by components, including the largest subunit of RNA polymerase IV (RPD1), required for the biogenesis of small RNA (sRNA)[1,2]. Because sRNAs in eudicots can move from cell to cell, exerting non-cell-autonomous effects on gene expression[3], we tested the cell-autonomous action of presumed sRNAs in maintaining repressed expression of a paramutant *purple plant1 (pl1)* allele (*Pl1-Rh*) by evaluating genetic mosaics for anthocyanin production related to RPD1 function. RPD1 dysfunction is associated with increased pigmentation in plants that are homozygous for repressed *Pl1-Rh alleles* (designated *Pl'*). By comparing pigmentation in hemizygous *rpd1* mutant sectors to that of adjacent non-mutant tissues we could evaluate the potential cell-autonomy of normal RPD1 function in repressing *Pl'*. If RPD1 function acts in a cell-autonomous manner, increased anthocyanin production would be expected throughout the sector, while non-cell-autonomous function would maintain pigment repression, at least at sector boundaries. One thousand nine plants (half heterozygous for the *rpd1-9* mutant allele) from irradiated seeds were evaluated in summer 2016. Of the 72 sectors greater than 0.5cm in width that were examined, 60 had sharp boundaries of dark pigment coincident with the cell-autonomous albino phenotype marking RPD1 loss. This result indicates that RPD1 actions - including the resulting sRNAs - are cell autonomous in their ability to induce and maintain *Pl'* states. This interpretation stands in contrast to results from grafting experiments in eudicots in which mobile sRNAs direct systemic epigenetic modifications[3].

Citations: 1. Brink 1958 *CSH Symp Quant Biol* **23**, 379 | **2.** Erhard 2009 *Science* **27**, 323 | **3.** Lewsey 2016 *PNAS* **9**, 113

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P384

Characterization of a candidate gene for *Ufo1*

(submitted by Jin Cui <juc326@psu.edu>)
Full Author List: Cui, Jin[1]; Wittmeyer, Kameron[1]; Tan, Qixian[1]; Xue, Weiya[2]; Lee, Tzuu-fen[3 4]; Meyers, Blake[3 5]; Chopra, Surinder[1]
[1] Department of Plant Science, The Pennsylvania State University, University Park, PA 16802
[2] Department of Plant Pathology and Environmental Microbiology, The Pennsylvania State University, University Park, PA 16802
[3] Department of Plant and Soil Sciences, University of Delaware, Newark, DE 19711
[4] DuPont Pioneer Hi-bred, P.O. Box 1000, Johnston, IA 50131-0184
[5] Division of Plant Sciences, University of Missouri, Donald Danforth Plant Science Center, St. Louis, MO 63132

Pericarp color1 (p1) is a Myb transcription factor involved in phlobaphene biosynthesis. *Unstable factor for orange1 (Ufo1)* is a dominant mutation that induces phlobaphene pigmentation in maize kernel pericarp. It was found that Ufo1 up-regulates p1 by reducing its DNA methylation level in its upstream enhancer sequence. Fine mapping results indicated that *Ufo1* gene is located in a 36 Mb region in chromosome 10 bin 3, but the low recombination in the mapping region has limited our effort to clone the gene. Based on our RNA-seq results, we've found few candidate genes (Gene 1 - 7) within the mapping region. Gene 7, an un-annotated gene is significantly up-regulated in the mutant vs. wild-type tissues. Real-time PCR confirmed that the candidate Gene 7 is highly up-regulated in the *Ufo1*-expressing (U-E) allele as compared to *Ufo1*-silent (U-S) allele and wild-type tissues. PacBio sequence analysis (Wittmeyer et al., unpublished) showed that the candidate gene contains a 4.7 kb CACTA-like transposable element at the 5' end of the gene's first intron. Bisulfite sequencing analysis showed that an upstream sequence within the CACTA element is highly de-methylated in U-E as compared to U-S and wild-type tissues. Small RNA-seq analysis also showed that this sequence contains significantly more siRNA of all sizes in the U-E tissues. These results show that our candidate gene might be epigenetically regulated and that the CACTA element might be essential for gene regulation.

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P385

Characterization of transgenerational epigenetic inheritance of sickly syndrome in a specific maize-teosinte backcross population

(submitted by Wei Xue <wxue22@wisc.edu>)
Full Author List: Xue, Wei[1]; Li, Qing[2]; Bilinski, Paul[3 4]; Yang, Liyan[1]; Ross-Ibarra, Jeffrey[3 4]; Springer, Nathan M[2]; Flint-Garcia, Sherry[5]; Doebley, John F[1]
[1] Department of Genetics, University of Wisconsin–Madison, Madison, Wisconsin, USA 53706
[2] Department of Plant Biology, Microbial and Plant Genomics Institute, University of Minnesota, Saint Paul, Minnesota, USA 55108
[3] Department of Plant Sciences, University of California, Davis, California, USA 95616
[4] Genome Center and Center for Population Biology, University of California, Davis, California, USA 95616
[5] USDA-ARS, Columbia, Missouri, USA 65211

Hybrids between maize and its wild relatives, the teosintes have been extensively used in studies of domestication and agriculture related traits. During these studies, a transgenerationally inherited sickly syndrome (morph-developmental abnormalities, and reduced vigor and fertility) was discovered three times independently when a particular teosinte (*Zea mays* ssp. *parviglumis*) from near Valle de Bravo, Mexico (VB) was used as the pollen parent for backcross (BC) to the recurrent female parents, inbred lines W22 or B73. The first signs of the sickly syndrome appear in the BC_1, however, it is much stronger from the BC_2 generation through the BC_7 generation. The sickly syndrome was not observed in these healthy controls: W22, B73, VB teosinte, a non-VB teosinte, and non-VB teosinte BCs. We used multiple approaches including genomics, methylomics and transcriptomics to identify the causative factor for sickly syndrome. Genotyping with genotype-by-sequencing (GBS) failed to identify any VB teosinte chromosome segment that is correlated with the sickly syndrome, suggesting that the sickly syndrome is caused by an epigenetic mechanism. Whole-genome bisulfite sequencing (WGBS) identified a few de novo methylation changes (different from both parents) in the BC_1 generation, and these were transmitted through later generations (BC_6). Many genes and small RNAs show consistently elevated expression in VB teosinte BCs plants with sickly syndrome, and a majority of these show altered methylation. Whole genome sequencing (WGS) results showed that multiple genomic sequences in VB teosinte BCs with sickly syndrome have increased copy number relative to controls. About half of these sequences align to differentially methylated regions (DMRs). Epigenetic changes appear to underlie the sickly syndrome, although the mechanism is unknown.

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P386

Chromatin modifications of repetitive DNA

(submitted by Jonathan Gent <gent@uga.edu>)
Full Author List: Gent, Jonathan I[1]; Lowry, Elizabeth G[1]; Higgins, David M[1]; Dawe, R Kelly[1 2]
[1] Department of Plant Biology, University of Georgia, Athens, GA, USA 30602
[2] Department of Genetics, University of Georgia, Athens, GA, USA 30602

The maize genome is famous for its repetitive DNA, from 100bp DNA transposons to megabase arrays of tandem repeats. While diverse forms of chromatin mark repetitive DNA, a unifying theme is transcriptional repression, or at least repression of the type of transcription that leads to mRNA. In some cases, multi-copy genes can be targeted for repression much like transposons or other repetitive DNA. The KinDr complex, a set of duplicated genes on the distal tip of the chromosome 10 variant Ab10, can acquire multiple repressed states that behave as epialleles. We are characterizing three distinct KinDr epialleles, all of which involve large increases in CG methylation in the promotor as well as increases in CHG methylation across the gene. RNA directed DNA methylation also appears to be involved in all three epialleles, though perhaps only for initiation. One of the three epialleles was initiated by a transgenic hairpin construct, but the resulting epiallele is heritable independent of the hairpin. In addition to characterizing the repression of the Kindr gene complex, we are examining whole genome patterns of chromatin modifications, DNA methylation, and small RNA to better understand how repetitive DNA and chromatin associated with repetitive DNA affect gene expression.

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P387

Chromosomal inversions caused by alternative transpositions in maize

(submitted by Sharu Paul Sharma <sharu@iastate.edu>)
Full Author List: Sharma, Sharu Paul[1]; Peterson, Thomas[1 2]
[1] Department of Genetics, Development and Cell Biology, Iowa State University, Ames, IA 50011
[2] Department of Agronomy, Iowa State University, Ames, IA 50011

Multiple copies of *Activator (Ac)* transposable elements present close together on a chromosome can undergo alternative transpositions, in which two termini of different *Ac, Ds or fAc* (fractured *Ac, Ac* with one end deleted) elements are involved. These events can cause genomic rearrangements like Inversions, Deletions, or Composite Insertions. We are interested in the structural changes in the genome that have some biological impact such as disrupted gene function, change in expression pattern, or formation of chimeric genes. Our group analyzes molecular structure of the genomic rearrangements caused by alternative transpositions between *p1* and *p2* genes present on chromosome 1. The p1 gene is expressed in the kernel pericarp, cob and silk; the p2 gene is expressed in anther and silk but not in pericarp. We use an allele termed p1-wwB54 which has an incomplete p1 gene with exons 1 and 2 deleted, thus the kernels are white. The p1-wwB54 allele also contains Ac and fAc elements with termini in reverse orientation which can undergo Reversed Ends Transposition (RET). From the B54 stock, we obtained inversions in which a *p1* enhancer is brought close to the *p2* gene promoter, thereby activating expression of *p2* in the kernel pericarp, resulting in red kernel color. This is a very effective screen for selection of inversion alleles. To date we have isolated 19 such cases of inversion alleles with red kernels; 10 cases were confirmed as inversions by PCR. The observed activation of *p2* expression in pericarp through a chromosome inversion indicates a position effect of the regulatory region of *p1* on *p2* expression. These results provide new insight into the mechanisms of genomic rearrangements and gene activation in the evolution of maize.

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P388

Comparison of centromere size and location in different maize accessions and their hybrids

(submitted by Na Wang <na.wang25@uga.edu>)
Full Author List: Wang, Na[1]; Gent, Jonathan I.[1]; Dawe, Kelly[1 2]
[1] Plant biology, 120 East Green Street, David Life Science Complex, University of Georgia, Athens, GA 30602, USA
[2] Genetics, 120 East Green Street, David Life Science Complex, University of Georgia, Athens, GA 30602, USA

Little is known about how centromere size and location are determined. Previous work in maize has revealed that centromere size is related to total genome size, and centromere locations are stably maintained in the absence of major genetic or physiological changes. Maize is a model organism for investigating these topics because of its genetic resources, including extreme diversity of centromere types between homologous chromosomes. Some of its centromeres are dominated by the tandem repeat CentC, but it also has complex centromeres with diverse types of retrotransposons. These complex centromeres enable assembly into reference genomes and mapping of short reads from chromatin immunoprecipitation (ChIP-seq). We are using ChIP-seq with CENH3, the maize centromeric histone H3 variant, to test the hypothesis that centromere position and size could be determined by trans-acting signals from homologous centromeres in hybrids with centromeres at non-equivalent positions. Our results suggested that each centromere can retain its parental position independently of it homolog, and hybridization did not affect centromere stability. We are also investigating dynamics of centromeric repetitive sequences, particularly the tandem repeat CentC, across Zea species to better understand relations between repetitive DNA and centromeres. It turned out that the abundance of CentC negatively correlated with the frequency of complex centromeres. These data provide new insights into the stabilization of maize centromere in the hybridization and how centromere repeats relate to centromere function.

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P389

Composite Insertions (CIs) and the evolutionary impact of Reversed-Ends Transposition (RET) in maize

(submitted by Weijia Su <weijia@iastate.edu>)
Full Author List: Su, Weijia[1]; Peterson, Thomas[1 2]
[1] Department of Genetics, Development and Cell Biology, Iowa State University, Ames, IA 50011
[2] Department of Agronomy, Iowa State University, Ames, IA 50011

Maize transposable elements are well known by their impact on gene expression and genome evolution. Our group is interested in *Ac/Ds* alternative transposition (AT) events. Unlike standard transposition which excises the two ends of a single transposon, AT engages the termini of two different nearby TE copies. One type of AT is termed Reversed-Ends Transposition (RET) which involves the reversely-oriented termini of two TEs on the same chromatid; for example, a complete *Ac* and a fractured *Ac (fAc)* inserted in the *p1* gene. Previous work in our lab showed that RET events can generate Tandem Direct Duplications (TDDs) and reciprocal deletions (Zhang et al., 2013), as well as novel structures termed Composite Insertions (CIs) which are formed by RET followed by DNA re-replication and repair (Zhang et al., 2014). By studying the effects of CIs at the maize *p1* and *p2* genes which regulate flavonoid pigment biosynthesis, we have found that CIs can alter gene expression by mobilizing an enhancer from *p1* to *p2*. To investigate the evolutionary impacts of RET, we are developing genome search algorithms to detect TDDs associated with flanking TEs. By analyzing TDDs genome wide, we will gain insight into the prevalence of Transposition-Induced Duplications (TIDs) compared to duplications induced by other mechanisms such as unequal crossing over.

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P390

Comprehensive analysis of lncRNA, mRNA, circRNA and miRNA expression uncovers a complex regulatory network that affects maize seed development

(submitted by Miaoyun Xu <xumiaoyun@caas.cn>)
Full Author List: xu, miaoyun[1]; zhang, min[1]; zhu, ming[1 2]; wang, lei[1]
[1] 1 Biotechnology Research Institute/The National Key Facility for Crop Gene Resources and Genetic Improvement, Chinese Academy of Agricultural Sciences, Beijing 100081, China
[2] School of Life Sciences, Anhui Agricultural University, Hefei 230036, China

Increasing evidence shows that crosstalk between RNAs mediated by shared miRNAs represents a novel layer of gene regulation, which plays important roles in plant development. Functional roles and regulatory mechanisms of lncRNAs and circRNAs as ceRNAs in plants, particularly in maize seed development, are unclear. In this study, we combined analyses of global transcriptomics and miRNA to generate a comprehensive resource focused on identifying key regulatory miRNA-mediated circuits at different stages of maize seed development. Consistently altered 17 lncRNAs, 840 mRNAs, 39 circRNAs and 35 miRNAs were selected and clustered among the three stages with Venn analysis. Then we performed a genome-wide analysis to investigate potential lncRNA-mediated ceRNA and circRNA-mediated ceRNA interplays based on “ceRNA hypothesis”. A lncRNA-associated and a circRNA-associated ceRNA network was constructed, respectively, by utilizing totally sample-matched 75 miRNA, 7 lncRNA, 15 circRNA and 219 mRNA expression profiles in seed. The results uncovered 7 novel lncRNAs and 15 circRNAs as potential functional ceRNAs. Their functions were predicted based on their competitive coding-gene partners by GO and KEGG biological pathway analysis. Functional analyses demonstrated that combined effects of multiple ceRNAs can have major impacts on general developmental and metabolic processes in maize seed. Furthermore, we found ceRNA interactions could sequentially and/or synergistically mediate the brassinolide signaling pathways during seed development.These findings provided a useful platform for uncovering novel mechanisms of maize seed development and may provide new insights into genetic engineering of crop production.

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P391

Dualism of *Muk* silencing: the transition between the transcriptional gene silencing and translational/post-translational inhibition during the *Muk*-induced silencing at various maize developmental stages

(submitted by Dafang Wang <wang2630@purdue.edu>)
Full Author List: Wang, Dafang[1]; Lisch, Damon[1]
[1] Purdue University; 915 W State St; West Lafayette; IN, USA 47907

Transposable Elements (TEs) comprise a large proportion of eukaryotic genomes including maize. In the face of the invasive TE populations, host species have evolved mechanisms that can effectively repress or silence TEs to preserve genome integrity and function, which include the siRNA-mediated silencing mechanism. We have demonstrated that in maize seedlings, *Muk* silences *MuDR* elements via transcriptional gene silencing (TGS). However, a distinct TE silencing pathway that functions at the translational level or post-translational was observed in imbibed embryos of maize seeds. The transition between the two distinct silencing pathways reflects different layers of defense against TE invasion during plant development. This observation raises questions concerning the developmental stage-dependent siRNA biogenesis and silencing regulation. For example, what components of which pathways are critical to the transition? Is there a novel silencing mechanism in the embryo? Why do host genomes adapt a novel transposon repression mechanism in the embryo? In this poster, we present the evidence for this transition of silencing mechanisms during plant development and discuss the possible means by which this transition is regulated.

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P392

Dynamic transposable element expression across development and stress in maize

(submitted by Sarah Anderson <sna@umn.edu>)
Full Author List: Anderson, Sarah N[1]; Stitzer, Michelle C[2]; Ross-Ibarra, Jeffery[2]; Springer, Nathan M[1]; Hirsch, Cory D[3]
[1] Department of Plant and Microbial Biology, University of Minnesota, St. Paul MN 55108
[2] Department of Plant Sciences, University of California Davis, Davis CA 95616
[3] Department of Plant Pathology, University of Minnesota, St. Paul MN 55108

Transposable elements (TEs) comprise a large portion of many eukaryotic genomes and are unique in their ability to replicate and move within genomes. The size of the maize genome is largely inflated by TE insertions, with the majority of these sequences derived from LTR retrotransposons. While a handful of specific TE families have been well characterized in maize, the majority of TEs have been difficult to characterize due to challenges associated with annotating, mapping, and assigning reads to the repetitive sequences common in TEs. Maize TE insertions have been associated with transcriptional changes in nearby genes, however little is known about the expression dynamics and regulation of the TEs themselves. We have utilized the new structural annotation of maize TEs in combination with a novel read assignment approach that accounts for the repetitive nature of TE sequences to assess RNA-seq datasets for TE expression on a per-family basis. We find substantial variation in both the proportion of the transcriptome derived from TEs and in the specific families with varied expression across development and following abiotic stress. We now have expanded opportunities to assess TE variation in a variety of genetic backgrounds and genomic contexts.

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P393

Exploring long noncoding RNAs in wheat wild relatives

(submitted by Alice Pieri <alice.pieri@santannapisa.it>)
Full Author List: Pieri, Alice[1]; Pè, Mario E.[1]; Bertolini, Edoardo[1]
[1] Institute of Life Sciences Scuola Superiore Sant'Anna; Pisa, Italy, 56127

The understanding of the complex bread wheat genome (*T. aestivum* AABBDD) can be facilitated by the study of its wild relatives. In this work we chose the donors of the A and D genomes, *T. urartu* and *Ae. tauschii*, to investigate the presence and function of long noncoding RNAs (lncRNAs), a class of regulators in animals and plants. To perform a comprehensive annotation of lncRNAs in the two progenitors, we collected 68 public RNA-Seq libraries generated from several organs and conditions. Reads were aligned to the respective reference genomes and both transcriptomes were *de novo* reconstructed. A stringent filtering pipeline based on lncRNAs main characteristics (length ≥ 200 nucleotides, ORFs ≤ 100 amino acids, no similarity with protein coding domains, housekeeping transcripts or small RNAs) was applied to the two sets of transcripts. We predicted 14,515 *T. urartu* and 20,908 *Ae. tauschii bona-fide* lncRNAs, showing features similar to those of other plant and animal counterparts, such as a reduced transcript length and number of exons. Thousands lncRNAs were significantly modulated in different organs and exhibited organ specific expression with a predominant accumulation in the spikes, sustaining the hypothesis of their critical biological role in reproductive organs. Interestingly, most of the organ-specific lncRNAs were found to be associated with transposable elements (TEs), suggesting a possible role of TEs in ncRNAs origin and differentiation. Although the majority of *T. urartu* and *Ae. tauschii* lncRNAs appears to be species-specific, we found 38 lncRNAs perfectly conserved between *T. urartu* and *Ae. tauschii*. In addition, we identified thousands of lncRNAs promoter sequences and lncRNAs transcripts conserved in the two progenitors and also in the A and D genome of bread wheat. Our work provides the first comprehensive atlas of wheat wild relatives lncRNAs and shed new light on their characteristics and conservation across different species.

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P394

Gene expression pattern of early maize embryo

(submitted by Dexuan Meng <xuan_0515@126.com>)
Full Author List: Meng, Dexuan[1]; Zhao, Jianyu[2]; Zhao, Cheng[3]; Luo, Haishan[1]; Xie, Mujiao[1]; Liu, Renyi[3]; Zhang, Xiaolan[2]; Jin, Weiwei[1]
[1] National Maize Improvement Center of China, Beijing, China,100193
[2] College of Horticulture,China Agriculture University, Beijing,China,100193
[3] Institute of Plant Stress Adverse Biology Research Center,Shanghai,China,201602

The embryo is responsible for transmitting genetic information to the next generation. Although several studies have shown the gene expression profiles of maize embryo between 9 DAP (days after pollination) and maturity stage, maize embryo at earlier stage before 9 DAP, especially the rod embryo stage, has not been well characterized. Using high-throughput RNA sequencing, we analyzed the allelic gene expression patterns of maize embryos from reciprocal crosses between inbred lines B73 and Mo17 at six time points (3DAP, 5DAP, 7DAP, 9DAP, 11DAP and 13DAP). A total of 9532 genes were found to be involved in early embryo development, including 3512 genes that were influenced by cross direction. Co-expression analysis provided further insights into the dynamics of gene expression during early embryo development. We found that 118 genes expressed in the embryo were imprinted at three-fold change compared to the expression of maternal and paternal alleles, among which 106 showed maternal preferential expression and 12 paternal preferential expressions. Some of the imprinted genes were continuously imprinted through six development stages. We found that 33 imprinted genes were newly and specifically imprinted in embryo, and 11 and 15 imprinting genes were conserved between maize and rice/Arabidopsis. Taken together, this study shed new light on our understanding of the gene expressed profiles and imprinted genes in the early development of maize embryo, and suggests that the imprinting phenomenon may be more extensive than previously thought.

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P395

Genotyping with UniformMu insertion elements

(submitted by James Whelan <jwhelan01@hamline.edu>)
Full Author List: Whelan, James[1]; Goering, Raeann[1]; Larsen, Siri[1]; Tan, Jia[1]; Makarevitch, Irina[1]
[1] 1536 Hewitt Ave, St Paul, MN 55104

Maize is a ubiquitous staple crop with approximately 90 million acres planted annually in the United States alone (United States Department of Agriculture). As the world's population grows, there is a continued exacerbation of global climates, resulting in a continued and growing need for new methods of corn manipulation to increase yield. In the process of identifying, analyzing and potentially manipulating genes of interest, all steps of the process are equally necessary and have the potential of presenting unique challenges. Objective: Identify mutagenic insertions with PCR genotyping through breeding of mutant stock. Methods: Mutant seed lines, 35 in total, each carried a Robertson Transposon, which is a highly exploitable insertional element and allows for immensely controllable gene breakage in, this case, a previously identified candidate gene. Several individuals were planted for each plant line and later had tissue collected after growth occurred. Genomic DNA was extracted using the Springer Lab Isolation protocol and flanking primers were developed using several bioinformatic tools. Along with these primers, a third primer was used for the mutagenic insertion. PCR was run on all isolated DNA and subsequent gel electrophoresis was performed to corroborate homozygosity or heterozygosity of plants. Results: A figure was developed, including a table which illustrates the number of plants, as well as plants that are homozygous for insertion. A specific gene of interest was used to model results as well as help with interpretation. Mutagenic insertions were successfully identified in many genomic sequences. Discussion: Despite approximately a 37% failure rate due to possibly non-working primers or other constraints, PCR amplification paired with gel electrophoresis was shown to be a novel way to identify the presence of a Robertson Transposon for the purpose of gene knockouts. This form of gene-breakage is undoubtedly a novel way to explore genetic, and ultimately, biological function.

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P396

High throughput identification of Ds insertion sites in maize using NGS

(submitted by Kazuhiro Kikuchi <kkikuchi@danforthcenter.org>)
Full Author List: Kikuchi, Kazuhiro[1]; Ahern, Kevin[2]; Vollbrecht, Erik[3]; Rong, Ying[1]; Kumar, Indrajit[1]; Brutnell, Thomas[1]
[1] Donald Danforth Plant Science Center, 975 N. Warson Road, St. Louis, MO 63132
[2] Boyce Thompson Institute, 533 Tower Road, Ithaca, NY 14853
[3] Iowa State University, Ames, IA 50011

Transposon tagging is an important tool for gene identification and characterization. However, mapping Ds sites in a large population can be very tedious and time consuming. We have developed a new high throughput methodology using Illumina sequencing that is very cost effective and efficient. As a case study, we analyzed 64 Ds insertion lines in which Ds insertion had been previously mapped. We developed a procedure based on a strategy used to map T-DNA insertions in A. thaliana (O'Malley et al, 2007, Nature Protocols, 2:2910). In brief, DNA was pooled, Ds flanking sequences amplified and PCR products amplified using Illumina adapters. Using this strategy, we were able to identify 40 out of 53 (75%) of known Ds insertion sites. We PCR validated a subset of these insertion and confirmed 26 of 27 Ds insertions tested. Moreover, we identified at least 139 potential new Ds insertions across these 64 lines. Our goal is to scale up to at least 10,000 Ds lines and map these Ds sites in a single Illumina flow cell. In summary, we successfully demonstrated this high throughput platform for Ds insertion site mapping in maize. These lines will serve as a platform for gene discovery and functional genomics in maize.

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P397

LTR-Retrotransposon dynamics in the Andropogoneae

(submitted by Dhanushya Ramachandran <dramacha@mix.wvu.edu>)
Full Author List: Ramachandran, Dhanushya[1]; Chalopin, Domitille[2]; McKain, Michael[3]; Kellogg, Elizabeth[3]; Bennetzen, Jeffrey[2]; Hawkins, Jennifer[1]
[1] Department of Biology, West Virginia University, Morgantown, WV, USA 26506
[2] Department of Genetics, University of Georgia, Athens, GA, USA, 30602
[3] Donald Danforth Plant Science Center, St. Louis, MO, USA, 63132

Flowering plants are known to undergo frequent hybridization and polyploidization.
Such genome merger events often result in TE mobilization, leading to local mutations and changes in genome size. Over time, most duplicated genomes return to a diploid state, and the TE expansion that occurred during the early stages of polyploidization may play a pivotal role in this genome restructuring during the diploidization process. The tribe Andropogoneae consists of over 1,200 morphologically diverse species including *Zea*, *Sorghum*, and sugarcane. Previous studies have found evidence for one whole genome duplication (WGD) event that occurred early in the origin of grasses (~70 mya), followed by numerous other duplication events including one approximately 5 mya from which the *Zea-Tripsacum* clade arose. The duplicated genomes of both species differentially responded to the diploidization process. Here, we describe TE activity, accumulation, and contribution to genome diversity in the *Zea-Tripsacum* clade. Although the allopolyploid ancestor of *Zea-Tripsacum* is extinct, we included two close diploid relatives for comparative analysis, which provide an opportunity to explore TE-associated events induced by hybridization and genome doubling from an evolutionary perspective. Utilizing comparative graph-based clustering of next generation sequence reads of eight panicoid grasses, we quantified the abundance estimates of various classes of genomic repeats. In all cases, LTR-retrotransposons were the most abundant repeats, but the genomes of each species were dominated by distinct families. Further, using molecular clock and phylogenetic analysis of repeat clusters that were shared among all species, we determined the extent to which TEs were activated in response to polyploidization, and their differential contribution to genome diversity in sister taxa that emerged from the same polyploidization event.

P398

LTR_retriever: a highly sensitive and accurate program for identification of LTR retrotransposons

(submitted by Shujun Ou <oushujun@msu.edu>)
Full Author List: Ou, Shujun[1]; Jiang, Ning[1]
[1] Michigan State University, East Lansing, MI, USA, 48910

Long-terminal repeats retrotransposons (LTR-RTs) are prevalent in most plant genomes. Identification of LTRs is important to study the temporal change of genome size and facilitate the de novo gene annotation. Homological identification of LTR regions is usually inefficient due to its species-specific feature. Benefit from the conserved structure of LTR-RTs, multiple highly sensitivities programs were published for de novo LTR recoveries. However, false positives could dominate the predictions and excessive curations are usually required. Here we report LTR_retriever, a multithreading empowered Perl program that could process LTR candidates and generate high-quality LTR libraries regardless of input or genome quality. Benchmark tests on model plant genomes indicate that LTR_retriever has achieved high levels of sensitivity, specificity, accuracy, and precision, which are 92.0%, 96.8%, 95.7% and 89.88% in rice, respectively, demonstrating significant improvements. LTR_retriever is also able to identify non-canonical LTRs accurately. A broad scan on 50 published plant genomes identified 57 non-TGCA type LTRs. The majority of these belong to the copia family with significantly shorter LTR regions. We also explored the feasibility of identifying intact LTR-RTs from self-corrected PacBio reads. The LTR library constructed from 40 thousand reads covering 4.5X of the Arabidopsis Ler-0 genome surpasses that constructed from the genome. Our package has demonstrated the highest performance with great flexibility for retrieving LTR-RT.

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P399

Mechanical chemical and CRISPR mutagenesis in perspective

(submitted by M. Gerald Neuffer <gneuffer@gmail.com>)
Full Author List: Neuffer, M. Gerald[1]; Chang, Ming[2]; Sheridaan, William[3]
[1] Uof Mo; Columbia, Mo; 65201
[2] 8915 Arcadia Avenue; San Gabriel Ca 91775
[3] U of N Dakota; Grand Forks N Dakota 58202

We have gathered thousands of mutants from various sources over the years from which we gained an understanding of the relative importance of the natural events; radiation, cytogenetic manipulation, transposon, chemicals, and the recently discovered exciting "CRISPR" DNA modulation on genetic and breeding research in Maize. All of these have provided insight into the biological phenomenon of mutation but only chemical mutagenesis has produced adequate numbers of dominant mutants to be effective in providing the genetic variations needed for evolution and crop improvement.
We can demonstrate that while natural phenomenon, over long time, have produced all the variations found in biology and how what we refer to as mechanical mutagenesis, has shown some promise of producing large numbers of mutants, but these actually were mostly recessive, dead end, destructive "KNOCKOUTs". Only chemical mutagenesis (EMS), which increased the natural frequency of all mutations by 1000 times, has produced positive dominant "KNOCKIN" cases which are essential for the variations mentioned above. We compare this with the new "CRISPR" modulation editing of DNA, which shows so much promise in human genetics and medicine but which to now has produced only a very few dominant mutants.
Because we know that corn and humans share disease genes with identical sequences we believe that an efficient way to make use of the amazing advances made in medical research by CRISPR technology would be to do CRISPR analysis of the 251+ dominant maize mutants and compare them directly to the human genome and thereby derive vital information unhampered by social consequences..

M. G. Neuffer, M. T. Chang, and W.F. Sheridan
Emeritus Prof, U of Mo: Retired Geneticist; BASF; Prof. U of N.Dakota

Funding acknowledgement: Corn Breeders Association

P400

Multiple maize reference genomes allow insight into intergenic transposable element evolution

(submitted by Michelle Stitzer <mcstitzer@ucdavis.edu>)
Full Author List: Stitzer, Michelle C[1]; Ross-Ibarra, Jeffrey[1 2]
[1] Center for Population Biology and Department of Plant Sciences, University of California, Davis, Davis, California
[2] Genome Center, University of California, Davis, Davis, California

Although the active movement of transposable elements (TEs) continues to generate phenotypes pivotal to the study of maize genetics, variation in TE content outside of loci generating large effect mutations is underappreciated, and even in existing inbred lines genome-wide TE differences have been recalcitrant to observation. Many methods have been developed in other species (e.g. Drosophila, rice, human) to characterize the presence and absence of TE copies using short read data, but the high TE content (85%), high paralogy, and high structural polymorphism of haplotypes in the maize genome has limited our confidence in distinguishing TE polymorphism outside of genic regions. With the growing availability of reference genomes of maize and wild relatives, we have the opportunity to use resolved haplotypes of assembled regions to validate TE polymorphism between individuals.
Using the pipeline we developed for annotating structural, full-length TE copies in the B73v4 reference genome, we annotate TEs in other references, and find from 90,000 to 150,000 TE copies. At face value, this matches expectations based on differing genome size between inbreds, but differences in genome assembly complicate this conclusion. We generate a metric of discordance between raw reads and assemblies to understand this difference. A large number of TE loci are present at orthologous positions between genomes, consistent with their age as measured by nucleotide substitutions within the TE, and large nested arrays of retrotransposons show presence/absence variation among genomes. For a highly fluid and dynamic genome like maize, multiple genomes allow greater understanding of the evolutionary forces at play in the coevolution between genomic parasites and their host. We anticipate that these insights into TE location can be used as a tool for future pan-genome investigations in maize.

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P401

Paramutation at the b1 locus is associated with RdDM activity at the paramutable B-I allele

(submitted by Maike Stam <m.e.stam@uva.nl>)
Full Author List: Lauss, Kathrin[1]; Lee, Tzuu-fen[2]; Bader, Rechien[1]; Hovel, Iris[1]; Gent, Jonathan[3]; Meyers, Blake[2 4]; Stam, Maike[1]
[1] University of Amsterdam, Swammerdam Institute for Life Sciences, Science Park 904 1098XH Amsterdam, The Netherlands
[2] Department of Plant and Soil Sciences, Delaware Biotechnology Institute, University of Delaware, Newark, DE 19711
[3] Department of Plant Biology, University of Georgia, Athens, Georgia 30602, USA
[4] Donald Danforth Plant Science Center, St. Louis, MO 63132, USA

Paramutation, the transfer of heritable silencing information between two alleles, results in a susceptible allele heritably acquiring the epigenetic profile of an inducing allele. Paramutation requires multiple factors of the RNA-directed DNA methylation (RdDM) pathway. This pathway involves small regulatory RNAs and is associated with the presence of asymmetric DNA methylation (mCHH). With paramutation at the b1 gene, a regulatory gene of the maize pigmentation pathway, the low expressed B' epiallele imposes its low transcription rate onto the high expressed B-I epiallele in trans, thereby changing B-I into B' in a mitotically and meiotically heritable manner. This change occurs with a 100% frequency. Seven tandem repeats of an 853 bp sequence, 100-kb upstream of the b1 gene are required for the in trans interaction and for high b1 expression. The B' repeats are DNA hypermethylated compared to the B-I repeats. We studied early steps in the paramutation process in maize embryos and found pronounced differences in DNA methylation patterns and small RNA levels between the regulatory repeats of the B' and B-I epiallele. Moreover, we identified an RdDM region (mCHH island) at the B-I repeats that might be involved in the initiation and spreading of DNA methylation into the unmethylated B-I repeat sequences during paramutation.

P402

Parent-of-origin effect *rough endosperm* mutants alter cellular development of the endosperm in maize

(submitted by Fang Bai <fbai001@ufl.edu>)
Full Author List: Bai, Fang[1]; Daliberti, Mary[1]; Bagadion, Alyssa[1]; Davenport, Ruth[1]; Xu, Miaoyun[2]; Li, Yubing[1]; Evans, Mathew M. S.[3]; Barbazuk, W. Brad[1]; Settles, A. Mark[1]
[1] University of Florida, Horticultural Sciences Department, Gainesville, FL, USA 32611
[2] Chinese Academy of Agricultural Sciences, BeiJing, P. R. China
[3] Carnegie Institution for Science, Stanford, CA, USA 94305

Parent-of-origin effect loci have non-Mendelian inheritance in which phenotypes are determined by either the maternal or paternal allele alone. In angiosperms, parent-of-origin effects can be caused by loci required for gametophyte development or by imprinted genes needed for seed development. Few parent-of-origin effect loci have been identified in maize even though there are a large number of imprinted genes known from transcriptomics. We screened *rough endosperm* (*rgh*) mutants for parent-of-origin effects using reciprocal crosses with inbred parents. Six *maternal rough endosperm* (*mre*) and three *paternal rough endosperm* (*pre*) mutants were identified with three *mre* loci mapped. When inherited from the female parent, *mre/+* seeds reduce grain-fill with a rough, etched, or pitted endosperm surface. Pollen transmission of *pre* mutants results in *rgh* endosperm as well as embryo lethality. Eight of the mutants had significant distortion from the expected one-to-one ratio for parent-of-origin effects. Linked markers for *mre1* , *mre2* , and *mre3* indicated that the mutant alleles have no bias in transmission. Histological analysis of *mre1* , *mre2* , *mre3* , and *pre*-949* showed altered timing of starch grain accumulation and basal endosperm transfer cell layer (BETL) development. The *mre1* locus delays BETL and starchy endosperm development, while *mre2* and *pre*-949* cause ectopic starchy endosperm differentiation. RNA-seq analysis from F1 crosses of *mre1* suggest a global increase in parent-of-origin biased gene expression suggesting the mutant delays endosperm development. Consistent with these transcriptomic data, *mre1* prolongs endosperm cell proliferation as assayed in cell culture. We conclude that many parent-of-origin effects in maize have incomplete penetrance of kernel phenotypes and that there is a large diversity of endosperm developmental roles for parent-of-origin effect loci.

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P403

Population genetic modeling of methylation variation in a natural teosinte population

(submitted by Jinliang Yang <jolyang@ucdavis.edu>)
Full Author List: Yang, Jinliang[1 2]; Li, Qing[3]; Doebley, John[4]; Springer, Nathan M.[3]; Ross-Ibarra, Jeffrey[1 5]
[1] Department of Plant Sciences, University of California, Davis, CA 95616, USA
[2] Department of Agriculture and Horticulture, University of Nebraska-Lincoln, Lincoln, NE 68588, USA
[3] Department of Plant Biology, Microbial and Plant Genomics Institute, University of Minnesota, Saint Paul, MN 55108, USA
[4] Laboratory of Genetics, University of Wisconsin, 425 Henry Mall, Madison, WI 5370, USA
[5] Center for Population Biology and Genome Center, University of California, Davis, CA 95616, USA

DNA methylation is a ubiquitous feature of plant genomes --- in maize, more than 40% of cytosines in the genome are methylated. Major progress has been made in describing the variation and functional consequences of DNA methylation across the genome as well as understanding the molecular mechanisms driving methylation. However, the evolutionary forces shaping the variation and landscape of DNA methylation are largely unknown. Here we conducted whole genome bisulfite sequencing (WGBS) on 20 individuals sampled from a natural population of the teosinte (*Zea mays* ssp. *parviglumis*) to investigate the evolutionary forces acting on methylation variation in nature. We identified co-methylated regions for each genome and used the boundaries to divide the genome into co-methylation blocks across the population. We inferred the methylation site frequency spectrum (mSFS) from co-methylation blocks, and implemented a Markov Chain Monte Carlo (MCMC) approach to fit the data to an explicit population genetic model incorporating forward and backward mutation as well as natural selection. Preliminary analyses suggest evidence of selection to maintain gene body methylation, but that much of the intergenic variation in methylation may be largely neutral.
Our results will enable a better understanding of the evolutionary forces acting on patterns of methylation in different contexts and functional regions of the genome.

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P404

Regulatory network analysis of maize small RNAs identifies modules associated with productivity traits

(submitted by Bosen Zhang <bszhang@illinois.edu>)
Full Author List: Zhang, Bosen[1]; Barber, Wesley T[1]; Li, Qing[1]; Hudson, Matthew E[1]; Moose, Stephen P[1]
[1] Department of Crop Sciences, University of Illinois at Urbana-Champaign, Urbana, Illinois, USA, 61801

Small RNAs (sRNAs) processed from non-coding transcripts are ubiquitous components for regulating plant development and maintain genome integrity by their control of gene expression. Plant sRNAs occur in three sizes of 21, 22, or 24 nucleotides that are generated by distinct pathways and function primarily in mRNA degradation, RNA-directed DNA methylation, and heterochromatin formation, respectively. Deep sequencing of small RNAs from 36 diverse maize inbred lines, and 27 hybrids derived from some of these inbred lines demonstrates extensive genome-scale diversity for accumulation of sRNAs, with evolutionary dynamics distinct from those captured by SNPs. Co-expression network analysis on these sRNA datasets revealed modules comprised of specific transposable element (TE) families, or associated primarily with either short (21 or 22nt) or long (24-nt) sRNAs. Some TE sRNA modules are highly correlated with vegetative biomass and seed yield, implying TE-sRNAs may contribute to variation for traits important to maize breeding. RNASeq data was generated from the same developing ear samples of the B73 hybrids that was used for sRNA sequencing, and network analysis also found modules showing strong positive or negative correlations with traits. Through integrated analysis of the sRNA and RNASeq datasets for the B73-derived hybrids, we discovered one module that included abundant 21-22nt sRNAs within inter-genic regions, AGO18a and MiR2118, each of which are components of the recently described phasiRNA pathway active during early pollen development. This phasiRNA module exhibited a strong negative correlation with grain yield, which intensified when hybrids were grown with limiting soil nitrogen. Our results demonstrate that presence-absence variation exists among maize genotypes for phasiRNA production in developing ear tissues, and suggest that phasiRNA activity is associated with reduced grain yields. Furthermore, application of co-expression network analysis to sRNA datasets can reveal novel insights into sRNA biological function and their potential contribution to maize productivity.

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P405

RNA-directed DNA methylation components affect *Mutator* transposon insertion preferences, spontaneous silencing and double strand break repair

(submitted by Meixia Zhao <zhao185@purdue.edu>)
Full Author List: Zhao, Meixia[1]; Lisch, Damon[1]
[1] Botany and Plant Pathology, Purdue University, West Lafayette, IN 47907, USA

Mutator elements are often used as mutagens in maize because of high rates of transposition activity and a preference for genic sequences, particularly the 5' end of genes. We wished to determine whether or not components of the RNA directed DNA methylation (RdDM) pathway, including *mop1* (*Mediator of paramutation1*), a putative RNA-dependent RNA polymerase, and *mop3* (*Mediator of paramutation3*), the predicted largest subunit of RNA polymerase IV (Pol IV), participate in *Mutator* activity and silencing. We found that in progeny of *mop1* and *mop3* mutants, insertions were shifted towards regions upstream of genes relative to progeny of their heterozygous siblings. These differences were enhanced in progeny of plants that had been mutant for two generations. In addition, we found that high copy active *Mutator* lines that were mutant for *mop1* and *mop3* were significantly less likely to undergo spontaneous silencing than their heterozygous siblings, suggesting that RdDM may play a role in this still unexplained phenomenon. Finally, we provide preliminary evidence that *MOP1* and *MOP3* are involved in efficient repair of *Mutator* induced somatic double-stranded breaks. Twenty-one and 22 nucleotide small RNAs were also observed in the *Mu* regions only in the *Mutator* line with activity, but not in any of the silenced lines.

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P406

Stability of a paramutant *pl1* allele is affected by a heritable cytoplasmic component

(submitted by Jay Hollick <hollick.3@osu.edu>)
Full Author List: Hollick, Jay B.[1 2 3]
[1] Department of Molecular Genetics, Center for RNA Biology, The Ohio State University, Columbus, OH 43210
[2] Department of Plant and Microbial Biology, University of California, Berkeley, CA 94720
[3] Institute of Molecular Biology, University of Oregon, Eugene, OR 97403

In contrast to the stability of most genetic factors, the maize *purple plant1* (*pl1*) allele *Pl1-Rhoades* (*Pl1-Rh*) exhibits meiotically heritable changes in gene regulation that are influenced by *trans*-homolog interactions. Such heritable epigenetic changes – known as paramutations – occur at specific alleles of four loci encoding transcription factors controlling flavonoid pigment biosynthesis and one locus affecting phytic acid levels. Transcriptionally repressed paramutant states of both *Pl1-Rh* (denoted *Pl′*) and *B1-Intense* (*B′*) alleles are somatically maintained by the action of 24-nucleotide RNA biogenesis components leading to working models in which allele-specific small RNAs (sRNAs) both mediate *trans*-homolog interactions and maintain paramutant states through a feed-forward RNA-directed DNA methylation-type mechanism.
Genetically non-equivalent sperm cells produced by TB-6Lc interchanges were used to evaluate the hypothesis that cytoplasmic components – possibly sRNAs – can induce or maintain paramutant *Pl′* states. Results show that paramutation induction at *pl1* requires locus-specific transmission indicating that no cytoplasmic, nor any endosperm-derived, agents facilitate paramutations. Previous communications regarding the stabilities of paramutant *red1* alleles (*R′*) and *Pl′* documented reversions to, or towards, higher expression states when *R′* or *Pl′* are maintained in hemizygous conditions. Anther pigment comparisons of segmental monoploid (*Pl′* / -) progeny generated using paramutant (*Pl′* / 6-B B-*Pl′*) and non-paramutant (*Pl* / 6-B B-*Pl*) pollen parents show that reversions only occur in plants receiving segmentally nullisomic sperm cells from 6-B B-*Pl* gametophytes. These data indicate that a cytoplasmic component of 6-B B-*Pl′* gametophytes, that is transmitted by sperm cells in the absence of the *pl1* locus itself, stabilizes paramutant *Pl′* states. This interpretation comports with a working model in which gametophytic sRNAs help maintain feed-forward loops of sRNA biogenesis occurring on chromosomal templates. Such heritable epigenetic agents might be expected to define a large component of phenotypic variation, especially in hybrids.

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P407

Transcriptional silencing of helitron-embedded miRNA target mimicry by RNA-directed DNA methylation (RdDM) and histone methylation

(submitted by Xinyan Zhang <zhan2168@purdue.edu>)
Full Author List: Zhang, Xinyan[1]; Lisch, Damon[1]
[1] Department of Botany and Plant Pathology, Purdue University; West Lafayette, IN, USA 47907

When the RdDM mutant, *mediator of paramutation1* (*mop1*), is maintained in a homozygous state for multiple generations, pleiotropic developmental defects are manifested in a progressive manner. Among these, the Sheath adaxilization (Shad) phenotype is often observed. This epimutant phenotype is reminiscent of the dominant *Rolled leaf1* mutant, which expresses a transcript that is no longer recognizable by miR166, and which exhibits adaxialization of leaf blade tissue. We found that the defect in the *mop1-shad* epimutant is associated with the derepression of a class of miR166-targeted lncRNAs that are embedded in helitrons. The epimutant phenotype is also associated with tissue-specific reduction reduction of miR166 and an increase in steady state levels of a number of transcripts that are normally targeted by this microRNA. We hypothesize that ectopic expression of these helitrons triggered by the loss of RdDM may act to induce miRNA target mimicry, reducing the overall pool of miR166. We suggest that RdDM is required to shape the boundary between heterochromatic helitrons and the flanking euchromatic regions, a hypothesis that is supported by the loss of repressive histone marks, DNA methylation and islands of 24 nt small RNAs at these boundaries in *mop1* mutants.

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P408

Transcriptome analysis reveal candidate genes for *Ufo1*

(submitted by Jin Cui <cuijinjincui4@gmail.com>)
Full Author List: Cui, Jin[1]; Wittmeyer, Kameron[1]; Tan, Qixian[1 2]; Xue, Weiya[1 3]; Lee, Tzuu-fen[4 5]; Meyers, Blake[4 6]; Chopra, Surinder[1]
[1] Department of Plant Science, The Pennsylvania State University, University Park, PA 16802
[2] Genewiz Inc., South Plainfield, NJ 07080
[3] Department of Plant Pathology and Environmental Microbiology, The Pennsylvania State University, University Park, PA 16802
[4] Department of Plant and Soil Sciences, University of Delaware, Newark, DE 19711
[5] DuPont Pioneer Hi-bred, P.O. Box 1000, Johnston, IA 50131-0184
[6] Division of Plant Sciences, University of Missouri, Donald Danforth Plant Science Center, St. Louis, MO 63132

Pericarp color1 (p1) is a Myb transcription factor involved in phlobaphenes biosynthesis. *Unstable factor for orange1 (Ufo1)* is a dominant mutation that induces phlobaphenes pigmentation in maize kernel pericarp. It was found that *Ufo1* up-regulates *p1* by reducing its DNA methylation in upstream enhancer sequence. Whole-genome bisulfite-seq and small RNA (smRNA-seq) results showed that *Ufo1* doesn't influence DNA methylation and siRNA production on a global scale. Fine mapping results indicated that *Ufo1* gene is located in about 36 Mb region on chromosome 10 bin 3, but the low recombination in the mapping region has limited our effort to clone the gene by map based methods. Based on our RNA-seq results, we've selected few candidate genes (Genes 1 - 7) within the mapping region. Gene 7, an un-annotated gene is currently our favorite target. It is significantly up-regulated in the mutant vs. wild-type plants. Real-time PCR assays also confirmed that the candidate Gene 7 is highly up-regulated in the *Ufo1* expressing (U-E) allele as compared to *Ufo1* -silent (U-S) allele and wild-type plants. PacBio sequence analysis (Wittmeyer et al., unpublished; see poster at this meeting) showed that Gene 7 contains a 4.7 kb CACTA transposable element at the 5' end of the first intron. Bisulfite sequencing analysis showed that an upstream sequence within the CACTA element is highly de-methylated in U-E as compared to U-S and wild-type plants. DNA methylation of *p1* intron II region in *P1-wr;Ufo1* plants also match that of CACTA DNA methylation in U-E plants indicating a common mechanism regulating *p1* and Gene 7 CACTA DNA methylation. Small RNA-seq analysis also showed that the CACTA upstream sequence contains significantly higher siRNA levels in the U-E plants. These results show that Gene 7 is epigenetically regulated and that the CACTA element may be essential for the regulation of dominant *Ufo1* phenotypes.

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P409

Two mutations define the *required to maintain repression10* locus affecting locus-specific paramutation

(submitted by Emily McCormic <mccormic.11@osu.edu>)
Full Author List: McCormic, Emily J.[1]; Hollick, Jay B.[2 3]
[1] Department of Molecular Genetics, The Ohio State University, Columbus, OH 43210
[2] Department of Molecular Genetics, Center for RNA Biology, The Ohio State University, Columbus, OH 43210
[3] Department of Plant and Microbial Biology, University of California, Berkeley, CA 94720

Paramutation at the *Pl1-Rhoades (Pl1-Rh)* allele of the *purple plant1 (pl1)* locus causes meiotically heritable changes in gene regulation that are influenced by trans-homolog interactions. Plants with an unrepressed *Pl1-Rh* alelle exhibit dark anther coloration, while plants with a repressed paramutant derivative (denoted *Pl'*) lack anther coloration. The transcriptional and post-transcriptional suppression of *Pl1-Rh* can be attributed to *required to maintain repression (rmr)* factors. We previously reported on eight different *rmr* factors identified by mutations induced by pollen treatment with ethyl methanesulfonate (EMS). At least six of these are required for the biogenesis of 24-nucleotide sRNAs that may direct cytosine methylation.
A new recessive mutation, ems073240, was tested for genetic complementation with other EMS mutations, including those representing other previously defined *rmr* loci. Complementation was found with all known mutations and *rmr*-type factors with the exception of *mop1*, for which no complementation data exists, and ems062986 which provisionally designated the *rmr10* locus. Molecular genotyping of mutant ems073240 plants indicated no co-segregation with sequences linked to the *mop1* locus consistent with the interpretation that ems073240 and ems062986 define a novel locus affecting paramutation. Results of genetic tests with the ems062986 mutation indicate that normal *rmr10* function is required for the meiotic maintenance of *Pl'* states but is not required for paramutation to occur at the *booster1* locus. Mapping these mutations using RNA-sequence data promises to identify a candidate gene whose encoded function explains its mechanistic role in the epigenetic repression of *Pl1-Rh*.

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P410

ZmEMFL1 is involved in regulating imprinted genes through H3K27me3 in Maize

(submitted by Chunlei Wang <wangchunlei3405@163.com>)
Full Author List: Wang, Chunlei[1]; Li, Xiaojie[1]; Dong, Xiaomei[1]; Li, Tong[1]; Lai, Jinsheng[1]
[1] Plant Genetics and Breeding National Maize Improvement Center, China Agricultural University, Beijing, China 100193

Polycomb group (PcG) proteins play a crucial role in many biological process by maintaining the repression of special sets of genes. By screening an EMS-mutagenized mutation pool of B73, we isolated an emf1 mutant in maize. AtEMF1 has been proposed to be a plant specific PcG protein and possesses a PRC1-like function. The emf1 mutant showed reduced plant height and seed size and delayed development in embryo and endosperm in maize. EMFL1 maintains the repression of cell division repressor genes through H3K27me3 modification above the whole genome level. In the endosperms of reciprocal cross homozygous emf1 mutants in different ecological backgrounds (B73 and Mo17), the number of paternal imprinted genes was obviously decreased compared with that in wild type, by reason of the lose of H3K27me3 modification on the maternal allele. Our study suggest that EMF1 participates in endosperm development and seed filling in maize through regulating genes expression level by H3K27me3 modification.

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P411

Effect of saline stress on the physiology and growth of maize hybrids and their related inbred lines

(submitted by Zi Shi <shizi_baafs@126.com>)
Full Author List: Luo, Meijie[1]; Zhao, Yanxin[1]; Song, Wei[1]; Shi, Zi[1]; Zhao, Jiuran[1]
[1] Maize Research Center, Beijing Academy of Agriculture and Forestry Sciences (BAAFS) Shuguang Garden Middle Road No.9, Haidian District Beijing, People's Republic of China,100097

Salinity is one major abiotic stress that restrict plant growth and crop productivity. In maize (Zea mays L.), salt stress causes significant yield loss each year. However, indices of maize response to salt stress are not completely explored and a desired method for maize salt tolerance evaluation is still not established. A Chinese leading maize variety Jingke968 showed various resistance to environmental factors, including salt stress. To compare its salt tolerance to other superior maize varieties, we examined the physiological and growth responses of three important maize hybrids and their related inbred lines under the control and salt stress conditions. By comparing the physiological parameters under control and salt treatment, we demonstrated that different salt tolerance mechanisms may be involved in different genotypes, such as the elevation of superoxide dismutase activity and/or proline content. With Principal Component Analysis of all the growth indicators in both germination and seedling stages, along with the germination rate, superoxide dismutase activity, proline content, malondialdehyde content, relative electrolyte leakage, we were able to show that salt resistance levels of hybrids and their related inbred lines were Jingke968 > Zhengdan958 > X1132 and X1132M > Jing724 > Chang7-2 > Zheng58 > X1132F, respectively, which was consistent with the saline field observation. Our results not only contribute to a better understanding of salt stress response in three important hybrids and their related inbred lines, but also this evaluation system might be applied for an accurate assessment of salt resistance in other germplasms and breeding materials

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Comparative proteomic analysis of two maize inbred lines upon long-term saline treatment

(submitted by Zi Shi <shizi_baafs@126.com>)
Full Author List: Luo, Meijie[1]; Zhao, Yanxin[1]; Shi, Zi[1]; Song, Wei[1]; Zhao, Jiuran[1]
[1] Maize Research Center, Beijing Academy of Agriculture and Forestry Sciences (BAAFS) Shuguang Garden Middle Road No.9, Haidian District Beijing, People's Republic of China,100097

Salt stress is one of the major abiotic stresses that limits maize grain yield throughout the world. To better understand the molecular mechanisms underlying salt tolerance in maize, a comparative proteomic analysis was employed to map the proteomics of seedling roots from the salt resistant genotype Jing724 and the salt sensitive genotype D9H under control and salinity conditions. Under salt treatment, the germination rate and growth parameters including seedling fresh/dry weight and shoot/root length of Jing724 were significantly higher than those of D9H at both germination stage and early seedling stage. Using the iTRAQ approach, 565 proteins were differentially regulated proteins (DRP), of which 89 were specific to Jing724 and 424 were specific to D9H. These DRPs were closely related to stress response, including defense, energy, metabolism and transport. In the salt stressed Jing724 root cells, DRPs related to 6-phosphogluconate dehydrogenase, NADPH producing dehydrogenase, ascorbate peroxidase, peroxidase, short-chain dehydrogenase reductase, USP family protein, glutamine synthetase, glutamate synthase and hydroxymethyltransferase were strongly overrepresented to facilitate energy management, maintenance of redox homeostasis, reducing ammonia toxicity, osmotic homeostasis regulation, stress defense, stress adaptation, gene transcription regulation and ion transport. The evaluation of superoxide dismutase activity, malondialdehyde content, relative electrolyte leakage and proline content were in agreement with the proteomic results. The findings not only shed light on the molecular network of salt tolerance in maize, but also provide valuable information for selection and breeding maize germplasms with enhanced tolerance to salinity.

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Mapping a major QTL for salt tolerance of mature maize plant in field based on SNP markers

(submitted by Zi Shi <shizi_baafs@126.com>)
Full Author List: Luo, Meijie[1]; Zhao, Yanxin[1]; Song, Wei[1]; Shi, Zi[1]; Zhao, Jiuran[1]
[1] Maize Research Center, Beijing Academy of Agriculture and Forestry Sciences (BAAFS) Shuguang Garden Middle Road No.9, Haidian District Beijing, People's Republic of China,100097

Salt stress seriously restricts plant growth and productivity. Maize is an important food and economic crop but also is a kind of salt sensitive crop. Identifying genetic factors governing salt tolerance is a sustainable solution to this problem for breeders. However, critical QTLs controlling salt tolerance of mature maize plant in field were yet not known. The aim of this study was to elucidate the main genetic factors contributing to salt tolerance of maize at mature plant stage. A diploid population (240 individuals) and 1317 SNP markers were used to produce a genetic linkage map covering 1462.05cM. A major QTL related to plant height of mature maize in saline field were detected on Chr1 with LOD score of 22.4, explaining 31.2% phenotypic variance. The QTL was verified by QTL mapping based on the trait of salt tolerance index, a ratio of plant height in saline field to those in normal field, and was co-localized the main QTL for seedling salt tolerance. By bioinformatics analysis, five candidate genes involved in ion homeostasis within the main QTL confidence interval were identified. This QTL location provides targets for marker assisted selection in salt tolerant maize lines breeding.

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P414

QTL mapping coupled with expression profiling identifies potential genes for aflatoxin resistance in corn

(submitted by Ramesh Dhakal <rdhakal06@gmail.com>)
Full Author List: Dhakal, Ramesh[1]; Subudhi, Prasanta K[1]
[1] LSU, Baton Rouge, LA, US, 70803

Aflatoxin, most potent carcinogenic secondary metabolite, produced by a A. flavus causes severe health hazard in human and livestock. Identification of potential candidate genes, and functional characterization host-plant resistance mechanism are the important steps for the development of new inbreds. A study was conducted for the identification of QTL resistance to aflatoxin in the population developed from B73 x Mp715. In addition, suppression subtraction hybridization (SSH) library was prepared to identify the differentially expressed genes (DEGs). Altogether 267 DEGs were identified, and reverse northern hybridization is used for expression study. Functional annotation of these DEGs revealed several genes associated to stress responses, signal transduction and disease resistance. These genes were placed in the linkage map using in-silico mapping. Fifty-six DEGs were located in and around the QTL region, and distributed in all chromosome except 6, 7, and 8. Twenty-six highly expressed genes were selected reverse transcription PCR (RT-PCR), and their expression was studied in seven inbreds (Mp715, Np719, Mp420, Mp313E, Mo18W, B73 and va35) at various time point after the fungal inoculation. Most of them were highly expressed in resistant inbreds as compared to susceptible. Quantitative real-time PCR (qPCR) was used to further validate expression pattern of few selected genes. Pathogenesis related protein (PR-4), DEAD-box RNA helicase, LRR family protein were highly expressed in resistant germplasm, most notably in Mp719. One of the important gene, PR-4, was located in the QTL region of chromosome 4 identified from our study. This integrated approach helped to identify the potential biological pathways and genes involved in resistance A. flavus and will be helpful for further study of host-plant resistance and host-pathogen interaction.

P415

From Medicine to Plant Sciences: the Promise of Computing on Phenotypic Descriptions for Predictive Phenomics

(submitted by Carolyn Lawrence-Dill <triffid@iastate.edu>)
Full Author List: Gkoutos, Georgios[1]; Lawrence-Dill, Carolyn J[2]
[1] Medical School, University of Birmingham IBERS and University of Aberystwyth, UK
[2] Iowa State University, Ames, IA 50011

We are adept at comparing genetic and genomic sequences. The collection of more such data promises increase our ability to determine gene function, discover and describe biological processes, and prioritize causative variants of interest that underlie disease response as well as other traits of interest across disciplines. Can we compare phenotypes and traits in a manner similar to how we compare genetic sequences? Here we present computational methodologies that demonstrate the power of computing across organized phenotypic descriptions. These methods facilitate the analysis of phenotype information across species, domains of knowledge, people, and machines. Examples of successful computation to recover known biological phenomena as well as to predict novel associations are presented here. Examples drawn from medical research as well as plant biology. Necessary changes in how we collect, analyze, and share data to enable these sorts of computations are presented.

Funding acknowledgement: NSF, NHGRI, BBSRC, USDA-ARS, ISU Plant Sciences Institute

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Crowdsourcing for Ground Truth: Using the Amazon Mechanical Turk to Collect Data for Image Analysis using Machine-Learning

(submitted by Carolyn Lawrence-Dill <triffid@iastate.edu>)
Full Author List: Siegel, Zachary[1]; Zhou, Naihui[1]; Zarecor, Scott[1]; Lee, Nigel[1]; Campbell, Darwin[1]; Nettleton, Dan[1]; Andorf, Carson[1 2]; Lawrence-Dill, Carolyn J[1]; Ganapathysubramanian, Baskar[1]; Friedberg, Iddo[1]; Kelly Jonathan[1]
[1] Iowa State University, Ames, IA 50011
[2] USDA-ARS, USA

We are adept at comparing genetic and genomic sequences. The collection of more such data promises increase our ability to determine gene function, discover and describe biological processes, and prioritize causative variants of interest that underlie disease response as well as other traits of interest across disciplines. Can we compare phenotypes and traits in a manner similar to how we compare genetic sequences? Here we present computational methodologies that demonstrate the power of computing across organized phenotypic descriptions. These methods facilitate the analysis of phenotype information across species, domains of knowledge, people, and machines. Examples of successful computation to recover known biological phenomena as well as to predict novel associations are presented here. Examples drawn from medical research as well as plant biology. Necessary changes in how we collect, analyze, and share data to enable these sorts of computations are presented.

Funding acknowledgement: NSF, NHGRI, BBSRC, USDA-ARS, ISU Plant Sciences Institute

Author Index

Participant List

Participant	Organization
Abraham, Jazmin	University of California Berkeley
Adhikari, Bibechana	Iowa State University
Aguilar Rangel, Mara	CINVESTAVIrapuato
Akaogu, Ijeoma	West Africa Center of Crop Improvement
Albert, Patrice	University of Missouri-Columbia
Albertsen, Marc	DuPont Pioneer
Alers Velazquez, Roberto	University of Toledo
Alexander, Martin	UC Berkeley PGEC
Alkhalifah, Naser	Iowa State University
Alves Ferreira, Marcio	Universidade Federal do Rio de Janeiro
Ames, Nicholas	University of Minnesota
Anderson, Alyssa	UC Berkeley PGEC
Anderson, Sarah	University of Minnesota
Andorf, Carson	USDA-ARS MaizeGDB
Anokye, Michael	LangebioCinvestav
Antonise, Rudie	KeyGene NV
Arp, Jennifer	University of Illinois Urbana-Champaign
Asaro, Alexandra	Donald Danforth Plant Science Center
Auger, Donald	South Dakota State University
Augustine, Robert	Washington University in St. Louis
Avila Bolivar, Luis	University of California Davis
Avramova, Viktoriya	Technical University of Munich
Baer, Marcel	University of Bonn

Participant	Organization
Bai, Fang	University of Florida
Balboni, Martina	University of Hamburg
Baldauf, Jutta	University of Bonn
Baldrich, Patricia	Donald Danforth Plant Science Center
Balint Kurti, Peter	USDA-ARS NC State University
Banan, Darshi	University of Illinois Urbana Champaign
Bao, Yan	Michigan State University
Barbazuk, Brad	University of Florida
Bartlem, Derek	KWS Gateway Research Center
Bartlett, Madelaine	University of Massachusetts Amherst
Bartos, Jan	Institute of Experimental Botany
Bass, Hank	FSU Tallahassee
Bastos Martins, Lais	North Carolina State University
Bauer, Eva	Technical University of Munich
Baxter, Ivan	USDA
Becraft, Phil	Iowa State University
Beissinger, Tim	USDA-ARS University of Missouri-Columbia
Bennetzen, Jeffrey	University of Georgia
Bertolini, Edoardo	Donald Danforth Plant Science Center
Best, Norman	Purdue University
Birchler, James	University of Missouri-Columbia
Blythe, Amanda	University of Missouri
Bohn, Martin	University of Illinois
Bommert, Peter	Universitt Hamburg
Boyer, Nate	University of Missouri-Columbia

Participant	Organization
Bradbury, Peter	USDA-ARS
Brandariz Zerboni, Sofia	University of Minnesota
Braud, Christopher	Donald Danforth Plant Science Center
Braun, David	University of Missouri-Columbia
Braun, Ian	Iowa State University
Bray, Adam	Danforth Center and Mizzou
Brohammer, Alex	University of Minnesota
Bruce, Wes	BASF Plant Science
Brunelle, Dale	University of North Dakota
Brutnell, Thomas	Enterprise RentACar Institute for Renewable Fuels
Bubert, Jessica	University of Illinois
Buckler, Edward	Cornell University
Burch, Merritt	South Dakota State University
Burdo, Brett	University of WisconsinMadison
Burgess, Diane	UC Berkeley
Cahill, James	DuPont Pioneer
Campbell, Darwin	Iowa State University
Campbell, Michael	Cold Spring Harbor Laboratory
Cannon, Ethy	USDA-ARS MaizeGDB
Cao, Yingying	Donald Danforth Plant Science Center
Caplan, Jeffrey	University of Delaware
Carneiro, Newton	Embrapa Maize and Sorghum
Chalivendra, Subbaiah	Louisiana State University
Chaluvadi, Srini	University of Georgia
Chamness, James	Cornell University

Participant	Organization
Charcosset, Alain	INRA
Chase, Christine	University of Florida
Chatterjee, Debamalya	Pennsylvania State University
Chatterjee, Mithu	Rutgers University
Chen, Angela	University of Illinois at UrabanaChampaign
Chen, Keting	Iowa State University
Chen, Noel	Novogene
Chen, Paoyang	Academia Sinica
Chen, ShuYun	Academia Sinica
Chen, Zhibin	Institute of Genetics and Developmental Biology
Chen, Zongliang	Waksman Institute of Microbiology
Chettoor, Antony	Carnegie Institution for Science
Chomet, Paul	NRGene
Chopra, Shara	Penn State University
Chopra, Surinder	Penn State University
Chotewutmontri, Prakitchai	University of Oregon
Chu, Kevin	Purdue University
Chuck, George	U.C. Berkeley Plant Gene Expression Center
Chumak, Nina	Department of Plant and Microbiology
Claeys, Hannes	Cold Spring Harbor Laboratory
Clark, Jan	University of North Dakota
Cody, Jon	University of Missouri-Columbia
Coelho, Carla	Donald Danforth Plant Science Center
Colasanti, Joseph	University of Guelph
Collier, Chad	Bionano Genomics

Participant	Organization
Conklin, Phillip	Cornell University
Conner, Kyle	University of Missouri-Columbia
Cooper, Julian	University of Illinois Urbana-Champaign
Corll, Jacob	Oakland University
Craig, Valerie	University of Guelph
Crisp, Peter	University of Minnesota
Cui, Jin	the Pennsylvania State University
Dai, Mingqui	Huazhong Agricultural University
Dannenhoffer, Joanne	Central Michigan University
Daskalska, Lora	University of Wisconsin-Madison
Davenport, Ruth	University of Florida
Davis, Carrie	Cold Spring Harbor Laboratory
Dawe, R. Kelly	University of Georgia
De JaegerBraet, Joke	University of Hamburg
de Leon, Natalia	University of Wisconsin-Madison
Deans, Natalie	The Ohio State University
Deason, Nicholas	Michigan State University
Del Valle Echevarria, Angel	University of Hawaii at Manoa
Dell Acqua, Matteo	Scuola Superiore Santanna
Della Penna, Dean	Michigan State University
Della Porta, Adriana	University of Florida
Demesa Arevalo, Edgar	Cold Spring Harbor Laboratory
DeValk, Craig	University of Wisconsin Madison
Dhakal, Ramesh	Louisiana State University
Dhungana, Singha	University of Missouri-Columbia

Participant	Organization
Diao, Xianmin	Institute of Crop Sciences
Diepenbrock, Christine	Cornell University
Ding, Yezhang	University of California
Dixon, Cullen	Penn State University
Doblas, Paula	UCSD
Dong, Jiaqiang	Waksman Institute
Dong, Zhaobin	Plant Gene Expression Center UC Berkeley
Dooner, Hugo	Rutgers University - Waksman Institute
Doust, Andrew	Oklahoma State University
Dresselhaus, Thomas	University of Regensburg
Du, Chunguang	Montclair State University
Dukowic Schulze, Stefanie	University of Minnesota
Duncan, Keith	Donald Danforth Plant Science Center
Dzievit, Matthew	Iowa State University
Eggleston, Bill	National Science Foundation
Ellis, Nathanael	Donald Danforth Plant Science Center
Emery, Marianne	University of Missouri-Columbia
Enders, Tara	University of Minnesota
Ersoz, Elhan	BensonHill Biosystems
Eudy, Douglas	Monsanto Company
Evans, Matthew	Carnegie Institution for Science
Eveland, Andrea	Donald Danforth Plant Science Center
Facette, Michelle	University of New Mexico
Falcon, Celeste	University of Wisconsin-Madison
Fan, Xiujun	PhytoAB Inc

Participant	Organization
Fang, Hong	Arkansas State University
Fei, Yi	China Agricultural University
Feldman, Max	Donald Danforth Plant Science Center
Feng, Wei	Carnegie Institution of Science
Fenton, Megan	Purdue University
Flint Garcia, Sherry	USDA-ARS
Fowler, John	Oregon State University
Frailey, Daniel	University of Georgia
Gaeta, Robert	Monsanto
Gage, Joseph	University of Wisconsin-Madison
Galic, Vlatko	Agricultural Institute Osijek
Gallavotti, Andrea	Waksman Institute
Ganal, Martin	TraitGenetics GmbH
Gao, Xiquan	Nanjing Agricultural University
Garcia, Nelson	Rutgers University
Gardiner, Jack	University of Missouri
Gardner, Candice	USDA
Gault, Christy	Cornell University
Ge, Fei	Waksman Institute of Microbiology Rutgers
Gent, Jonathan	University of Georgia
Giarratano, Rocky	University of Georgia
Gibson, Ryan	Dow AgroSciences
Gingeras, Thomas	Cold Spring Harbor Laboratory
Glazebrook, Jane	University of Minnesota
Glowinski, Anna	University of Missouri-Columbia

Participant	Organization
Goering, Rae	Hamline University
Goldshmidt, Alexander	Monsanto
Gomez Cano, Fabio	Ohio State University
Gonzalez, Eric	LANGEBIO
Gordon Kamm, Bill	DuPont Pioneer
Gore, Michael	Cornell University
Govindarajulu, Rajanikanth	West Virginia University
Graham, Nat	University of Missouri Columbia
Gray, John	University of Toledo
Griffin, Brianna	Florida State University
Gronevelt, J. Paige	Oakland University
Grotewold, Erich	The Ohio State University
Gumber, Hardeep	Florida State University
Guo, Tingting	Iowa State University
Guo, Wei	Purdue University
Guthrie, Katherine	University of Missouri Columbia
Gyawali, Abiskar	University of Missouri
Hacisalihoglu, Gokhan	Florida AM University
Hajmohammadi, Solmaz	AlgorithmSoftware Developer
Hake, Sarah	USDA-ARS
Han, Fangpu	Chinese Academy of Sciences
Han, Mengqiao	University of Illinois at Urbana-Champaign
Hannah, L. Curtis	University of Florida
Harkess, Alex	Danforth Center
Harper, Lisa	USDA-ARS MaizeGDB

Participant	Organization
Hartwig, Thomas	Carnegie Institution for Science
Hattery, Travis	Iowa State University
Hawkins, Jennifer	West Virginia University
Hayden, Daniel	University of Oklahoma
He, Mingze	Iowa State University
He, Xiujing	Sichuan Agricultural University
Heller, Nicholas	University of Illinois
Henderson, Ashley	West Virginia University
Hey, Stefan	University of Bonn
Hiatt, Evelyn	Kentucky Wesleyan College
Higgins, David	University of Georgia
Hill Skinner, Sarah	Iowa State University
Hirsch, Candice	University of Minnesota
Hochholdinger, Frank	University of Bonn
Hodge, John	Oklahoma State University
Holding, David	University of Nebraska
Hollick, Jay	The Ohio State University
Holmes, Mark	University of Minnesota
Hood, Elizabeth	Arkansas State University Biosciences Institute
Hopkins, Corrie	Monsanto Company
Hsu, FeiMan	University of Tokyo
Hu, Hao	Oklahoma State University
Hu, Kun	Sichuan Agricultural University
Hu, Ying	Kansas State University
Huang, Cheng	China Agricultural University

Participant	Organization
Huang, Wei	China Agricultural University
Huffaker, Alisa	UC San Diego
Hufford, Matthew	Iowa State University
Hufnagel, David	Iowa State University
Hunter, Charles	USDA-ARS
Ibarra Reyes, Elsa	CINVESTAV Irapuato
Islam, Md Soliman	University of Missouri Columbia
Ivleva, Natalia	Monsanto
Jackson, David	Cold Spring Harbor Laboratory
Jamann, Tiffany	University of Illinois
Janzen, Garrett	Iowa State University
Jaqueth, Jen	Pioneer
Je, Byoung Il	Cold Spring Harbor Laboratory
Jia, Guanqing	Institute of Crop Sciences
Jia, Shangang	University of Nebraska Lincoln
Jiang, Hui	Donald Danforth Plant Science Center
Jiang, Nan	Ohio State University
Jiao, Yinping	Cold Spring Harbor Laboratory
Jin, Shan	The Pennsylvania State University
Jinga, Stephen	University of Illinois
Johal, Guri	Purdue University
Johnson, Adam	University of Missouri
Johnson, Eden	University of Missouri Columbia
Joshi, Trupti	University of Missouri-Columbia
Julius, Ben	University of Missouri-Columbia

Participant	Organization
Jung, Mark	DuPont Pioneer
Kabahuma, Mercy	Iowa State University
Kaczmar, Nicholas	Cornell University
Kadam, Dnyaneshwar	University of Minnesota Twin Cities
Kaeppler, Heidi	University of Wisconsin
Kaeppler, Shawn	University of Wisconsin-Madison
Kantanka, Sarfo	Florida AM University
Karlen, Steven	University of Wisconsin Madison
Kaur, Amanpreet	Purdue University
Kazic, Toni	University of Missouri
Kelliher, Timothy	Seeds Research Syngenta Crop Protection
Kellogg, Elizabeth	Donald Danforth Plant Science Center
Kelly, Jacob	BYU
Kermicle, Jerry	University of Wisconsin
Kessler, Sharon	Purdue University
Khangura, Rajdeep	Purdue University
Kianian, Penny	University of MinnesotaTwin Cities
Kikuchi, Kazuhiro	Donald Danforth Plant Science Center
Kimble, Ashten	University of Missouri-Columbia
Klein, Harry	University of Massachusetts Amherst
Kleinmanns, Julia	UCSD
Kleintop, Adrienne	Delaware Valley University
Kloiber Maitz, Monika	KWS SAAT SE
Knauer, Steffen	Cold Spring Harbor Laboratory
Ko, Dae Kwan	Michigan State University

Participant	Organization
Koch, Karen	University of Florida
Kokulapalan, Wimalanathan	Iowa State University
Kol, Guy	NRGene
Kolkman, Judith	Cornell University
Kono, Thomas	University of Minnesota
Kremling, Karl	Cornell University
Kriz, Al	Bayer
Krueger, Kyle	University of Wisconsin-Madison
Kumar, Dhinesh	Donald Danforth Plant Science Center
Kumar, Indrajit	Donald Danforth Plant Science Center
Kusmec, Aaron	Iowa State University
Lai, Xianjun	University of Nebraska-Lincoln
Lal, Shailesh	Oakland University
Lamb, Jonathan	Monsanto
Lamkey, Kendall	Iowa State University
Larkins, Brian	University of Nebraska
Larsen, Siri	Hamline University
Larsson, Sara	Pioneer
Larue, Huachun	Monsanto
Laspisa, Daniel	University of Hawaii at Manoa
Lauter, Nick	USDA-ARS
Lawrence Dill, Carolyn	Iowa State University
Leach, Kristen	University of Missouri-Columbia
Lee, DongYeon	Donald Danforth Plant Science Center
Lee, Elizabeth	University of Guelph

Participant	Organization
Leiboff, Samuel	UC Berkeley PGEC
Lennon, Jill	DuPont Pioneer
Leonard, April	DuPont Pioneer
Li, Bailin	DuPont Pioneer
Li, Mao	Danforth Plant Science Center
Li, Pinghua	Shandong Agricultural University
Li, Qi	Institute of Plant Physiology Ecology
Li, Weiya	China Agricultural University
Li, Xianran	Iowa State University
Li, Xiaowei	China Agricultural University
Li, Xuexian	China Agricultural University
Li, Yubin	Biotechnology Research Institute
Li, Zhaoxia	Iowa State University
Li, Zhi	University of Minnesota
Liang, Zhikai	University of NebraskaLincoln
Lin, Guifang	Kansas State University
Lin, Meng	Cornell University
Lindskoog, Zachary	Cornell University Plant Transformation Facility
Lipka, Alexander	UIUC
Lisch, Damon	Purdue University
Liu, Chenxu	China Agricultural University
Liu, Han	China Agriculture University
Liu, Hongjun	Sandong Agricultural University
Liu, Jianing	University of Georgia
Liu, Jie	Institute of Genetics and Developmental Biology Ch

Participant	Organization
Liu, Jingyan	China Agricultural University
Liu, Lei	Cold Spring Harbor Laboratory
Liu, Peng	University of Florida
Liu, Qiujie	Waksman Institute of Microbiology
Liu, Ruixiang	JAAS Iowa State University
Liu, Sanzhen	Kansas State University
Liu, Xue	Waksman Institute of Microbiology
Liu, Yalin	Chinese Academy of Sciences
Liu, Yang	Chinese Academy of Sciences
Liu, Zhengbin	Danforth Plant Science Center
Longstaff, Muriel	Brigham Young University
Lopez, Miriam	USDA-ARS
Lor, Vai	University of Wisconsin-Madison
Lorant, Anne	UC Davis
Lowdon, Rebecca	Monsanto
Lu, Fei	Cornell University
Lubberstedt, Thomas	Iowa State University
Lukens, Lewis	University of Guelph
Lunde, China	UC Berkeley PGEC
Ma, Xiaoli	University of Tuebingen
Madlambayan, Gerard	Oakland University
Madzima, Thelma	University of Washington Bothell
Magallanes Lundback, Maria	Michigan State University
Magwire, Michael	Syngenta
Makarevitch, Irina	Hamline University-Biology Department

Participant	Organization
Man, Jarrett	University of Mass-Amherst
Manchanda, Nancy	Iowa State University
Manching, Heather	University of Delaware
Manley, Bethan	University of Cambridge
Marcon, Caroline	University of Bonn
Marino, Thiago	NCSU
Martin, Barry	CiBO Technologies
Mathioni, Sandra	Danforth Center
Matschi, Susanne	University of California, San Diego
Matthes, Michaela	University of Missouri Columbia
Mazaheri, Mona	University of Wisconsin-Madison
McCarty, Donald	University of Florida
McCaw, Morgan	University of Missouri-Columbia
McClish, Allison	The Ohio State University
McCombie, W. Richard	Cold Spring Harbor Laboratory
McCormic, Emily	The Ohio State University
McCubbin, Tyler	University of Missouri
McFarland, Bridget	Iowa State University
McFarland, Frank	University of Wisconsin Madison
McGinnis, Karen	Florida State University
McLoughlin, Fionn	Washington University in St. Louis
McMullen, Madeline	Iowa State University
McNellie, James	Iowa State University
McNinch, Colton	Iowa State University
McReynolds, Maxwell	Iowa State University

Participant	Organization
McSteen, Paula	University of Missouri
Meeley, Robert	DuPont Pioneer
Mei, Wenbin	University of California
Mejia Guerra, Maria	Cornell University
Mendoza, Janette	University of New Mexico
Meng, Dexuan	National Maize Improvement Center of China
Mertz, Rachel	University of Missouri-Columbia
Messing, Joachim	Rutgers University
Meyers, Blake	Donald Danforth Plant Science Center
Mezmouk, Sofiane	KWS SAAT SE
Miao, Chenyong	University of Nebraska-Lincoln
Michel, Kathryn	University of Wisconsin-Madison
Miles, Nicholas	University of North Texas
Miller, Kari	Danforth Plant Science Center
Miller, Kathleen	University of Wisconsin-Madison
Milsted, Claire	University of Minnesota
Minow, Mark	University of Guelph
Moose, Steve	University of Illinois
Morales, Laura	Cornell University
Moran Lauter, Adrienne	USDA-ARS
Morasi De Sousa, Sylvia	Embrapa Milho e Sorgo
Mu, Qi	Iowa State University
Mudunkothge, Janaki	University of Florida
Muehlbauer, Gary	University of Minnesota
Multani, Dilbag	DUPONT PIONEER

Participant	Organization
Muszynski, Michael	University of Hawaii at Manoa
Nannan, Liu	Huazhong Agricultural University
Nannas, Natalie	University of Georgia
Neelakandan, Anjanasree	Iowa State University
Nelissen, Hilde	VIB UGent
Nelms, Brad	Stanford University
Nelson, Rebecca	Cornell University
Neuffer, M. Gerald	University of Missouri-Columbia
Neuffer, Rosemary	
Neuman, David	NRGene
Newton, Kathleen	University of MissouriColumbia
Nguyen, Hung	University of Missouri
Niculaes, Claudiu	Technical University of Munich
Noshay, Jaclyn	University of Minnesota
Novak, Stephen	Dow AgriSciences
Okagaki, Ron	University of Minnesota
Okamuro, Diane	NSFBIOIOS
Oppenheimer, Jara	University of Hamburg
Otegui, Marisa	University of Wisconsin-Madison
Ott, Alina	Iowa State University
Ou, Shujun	Michigan State University
Ouma, Zachary	Ohio Sate University
Ouzunova, Milena	KWS SAAT SE
Pardo, Jeremy	Cornell University
Park, Woojun	Kansas State University

Participant	Organization
Parvathaneni, Rajiv Krishna	Danforth Center
Pauwels, Laurens	VIBUGent Center for Plant Systems Biology
Pawlowski, Wojtek	Cornell University
Pedersen, Sarah	Iowa State University
Pei, Yuanrong	Institute of Genetics and Developmental Biology
Penghao, Wu	Xinjiang Agricultural University
Percifield, Ryan	West Virginia University
Peters, David	USDA
Peters, Jason	Southern University
Petersen, Michael	Wisconsin Crop Innovation Center UW Madison
Peterson, Thomas	Iowa State University
Pieri, Alice	Scuola Superiore Sant Anna Pisa
Planta, Jose	Rutgers
Poretsky, Elly	University of California San Diego
Portwood, John	USDA-ARS
Posekany, Tes	Iowa State University
Praud, Sebastien	Biogemma
Presting, Gernot	University of Hawaii
Qiao, Pengfei	Plant Biology
Ramachandran, Dhanushya	West Virginia University
Rasmussen, Carolyn	University of California
Rath, Mary	Arkansas State University
Ream, Thomas	Monsanto
Rellanlvarez, Ruben	Langebio
Ren, Jiaojiao	Iowa State University

Participant	Organization
Ren, Ying	University of Nebraska Lincoln
Renk, Jonathan	University of Wisconsin-Madison
Rhodes, Brian	University of Illinois Urbana-Champaign
Ribeiro, Camila	University of Florida
Ricci, William	University of Georgia
Rice, Brian	University of Illinois
Rice, Elena	Monsanto company
Rios Acosta, Lorena	University of Illinois
Ritchie, Steven	Agrivida
Robil, Janlo	University of Missouri Columbia
Rodriguez, Jonas	University of Wisconsin-Madison
Romay, Cinta	Cornell University
Rong, Ying	Donald Danforth Plant Science Center
Ross, Edward	University of Illinois Urbana-Champaign
Ross Ibarra, Jeffrey	University of California, Davis
Runcie, Daniel	UC Davis
Rupe, Mary	DuPont Pioneer
Sachs, Marty	Maize Genetics COOP Stock Center
Salvi, Silvio	University of Bologna
Salvo, Stella	Monsanto
Sampson, Elizabeth	University of Minnesota
Sanclemente, Maria Angelica	University of Florida
Saponaro, Philip	University of Delaware
Satterlee, James	Cornell University
Savadel, Savannah	Florida State University

Participant	Organization
Scanlon, Michael	Cornell University
Schaefer, Chris	DuPont Pioneer
Schaefer, Robert	University of Minnesota
Schaeffer, Mary	MaizeGDB
Scheidegger, Adam	University of North Dakota
Schmelz, Eric	UCSD
Schnable, James	University of Nebraska-Lincoln
Schnable, Patrick	Iowa State University
Schneider, Kevin	UH Manoa
Scott, Paul	USDA-ARS
Sebastian, Jose	Carnegie Institution for Science
Sekhon, Rajandeep	Clemson University
Sen, Sidharth	University of Missouri-Columbia
Septiani, Popi	Scuola Superiore Sant Anna Pisa
Settles, Mark	University of Florida
Sharma, Sharu Paul	Iowa State University
Shenstone, Esperanza	University of Illinois
Sher, Andrew	UCSD
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Shi, Xiaowen	University of Missouri
Shrestha, Vivek	University of Missouri-Columbia
Shuler, Stacie	University of Wisconsin-Madison
Shyu, Christine	Donald Danforth Plant Science Center
Sidorenko, Lyudmila	Dow Agro Sciences
Siebert, Amy	Oakland University

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Singh, Akanksha	Purdue University
Singh, Archana	University of Kentucky
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Smith, Laurie	University of California, San Diego
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Stein, Joshua	Cold Spring Harbor Laboratory
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Stinard, Philip	USDA-ARS Maize Genetics Stock Center
Stitzer, Michelle	University of California
Strable, Josh	Cornell University
Struttmann, Joseph	University of Missouri
Studer, Anthony	University of Illinois Urbana-Champaign
Su, Handong	Institute of Genetics & Developmental Biology Chin

Participant	Organization
Su, Weijia	Iowa State University
Sun, Xiaopeng	HuaZhong Agriculture University
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Thompson, Addie	Purdue University
Thompson, Beth	East Carolina University
Tian, Xiaolong	China Agricultural University
Timmermans, Marja	Cold Spring Harbor Laboratory
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Tracy, William	University of Wisconsin-Madison
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Wang, Jinyu	Iowa State University
Wang, Kan	Iowa State University

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Wang, Rachel	Academia Sinica
Wang, Ruifeng	China Agricultural University
Wang, Xuewen	University of Georgia
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Warburton, Marilyn	USDA ARS CHPRRU
Ware, Doreen	USDA-ARS Cold Spring Harbor Laboratory
Warman, Matthew	Oregon State University
Washburn, Jacob	University of Missouri
Weber, Blaise	University of Amstedam
Weber, David	Emeritus
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Wittmeyer, Kameron	Pennsylvannia State University

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Wu, Jingrui	DuPont Pioneer
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Wu, Yongrui	Shanghai Institute of Plant Physiology Ecology
Xia, Jingnu	University of Illinois - Urbana Champagne
Xiang, Yanli	Huazhong Agricultural University
Xiao, Yingjie	Huazhong Agricultural University
Xiao, Yingni	China Agricultural University
Xiao, Yuguo	Brigham Young University
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Xu, Xiaosa	Cold Spring Harbor Laboratory
Xue, Wei	University of Wisconsin-Madison
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Yamasaki, Masanori	Kobe University

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Yan, Lang	University of Nebraska-Lincoln
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Yang, Hua	University of Missouri-Columbia
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Yu, Yunqing	Donald Danforth Plant Science Center
Yuan, Jing	Institute of Genetics and Developmental Biology
Yuan, Ningning	Institute of Plant Physiology Ecology
Zanouny, Abdalla	University of Wisconsin Madison
Zebosi, Brian	Iowa State University
Zeng, Shuai	University of Missouri-Columbia
Zhan, Ross	Purdue University
Zhang, Bosen	University of Illinois -Urbana Champaign
Zhang, Jing	Institute of Genetics and Developmental Biology
Zhang, Quan	Donald Danforth Plant Science Center
Zhang, Xia	Chinese Academy of Agricultural Sciences
Zhang, Xiaoyue	University of Illinois at Urbana Champaign
Zhang, Xinyan	Purdue University

Participant	Organization
Zhang, Yang	University of Nebraska Lincoln
Zhang, Zhiyong	Waksman Institute of Microbiology
Zhao, Changzeng	University of Missouri
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Zhao, Li	Institute of Genetics & Developmental Biology Chin
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Zhao, Xiangyu	Shandong Agricultural University
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Zheng, Qi	Sichuan Agricultural University
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Zhou, Adele	Cornell University
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Zhou, Yan	Iowa State University
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