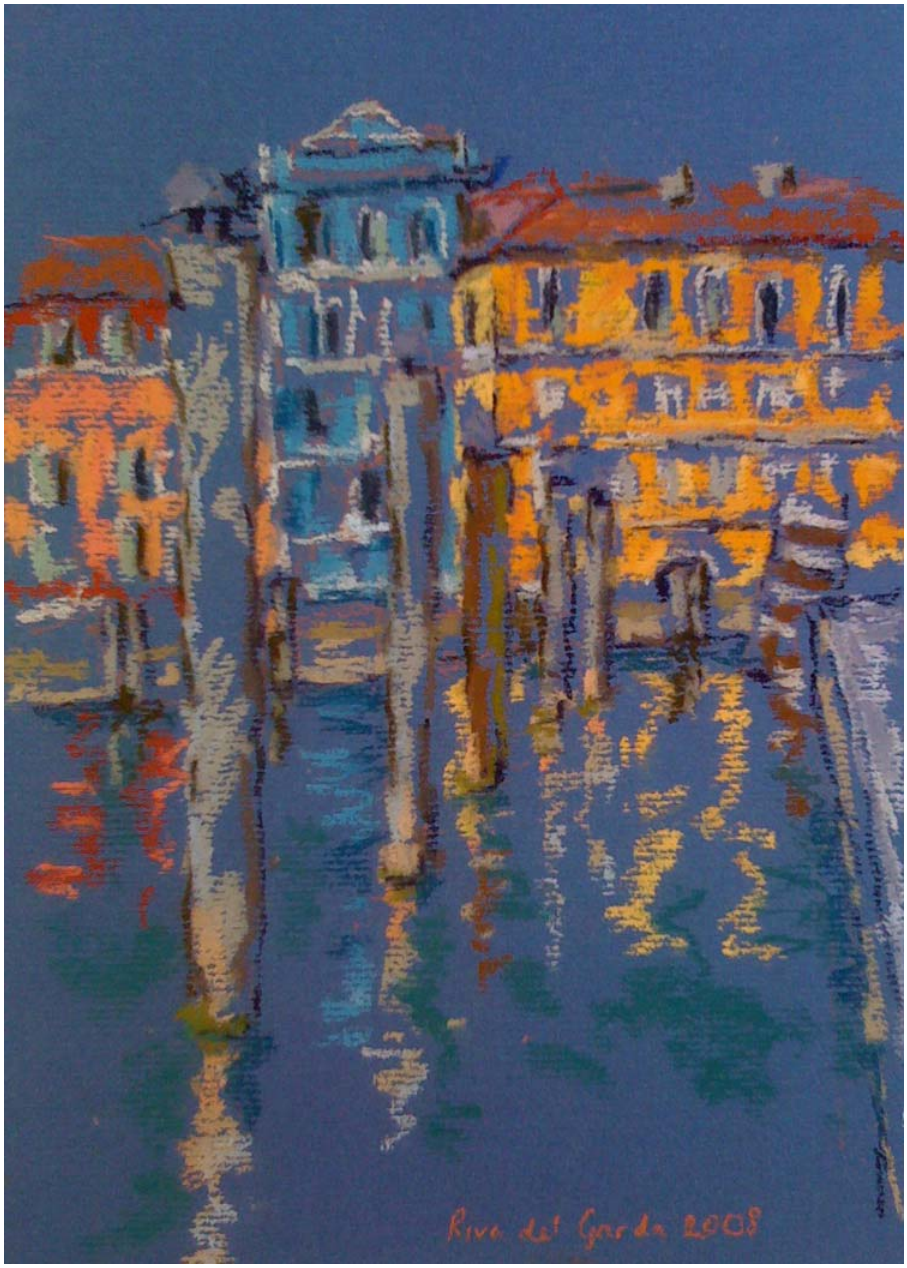

52nd Annual Maize Genetics Conference

Program and Abstracts

March 18 – March 21, 2010

Congress Centre
Riva del Garda (Trento), Italy

This conference received financial support from:

National Science Foundation | Monsanto | Pioneer Hi-Bred International, Inc. A DuPont Company | Syngenta | Dow Agrosciences | BASF Corporation Agricultural Products | National Corn Growers Association and State Partners | Keygene | Biogemma | CRA – Consiglio Per La Ricerca E La Sperimentazione In Agricoltura | KWS | Ministero Delle Politiche Agricole Alimentari E Forestali | Universita Degli Studi Di Milano | Limagrain | Provincia di Autonoma Trento

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DI MILANO

We thank these contributors for their generosity!

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Participants 187

Cityscape by Bianca ALberti, Milano, Italia

Cover art by Enrico Coen, John Innes Centre, England

General Information

Registration
Registration is Thursday March 18 from 3:00 – 6:00 PM in the Riva Conference Center reception area.

Meals
Breakfast is on your own. All the hotels include a continental breakfast with the cost of your lodging. All other meals will be served buffet style in the Riva Conference Center Palameeting area during the hours listed in the Program. Refreshments (coffee, tea and soft drinks) are available at no charge during the session breaks and poster sessions, as listed in the program.

Talks and Posters
All regular session talks, the Genome Update session and the Community Discussion will be presented in the Riva Conference Center auditorium (room 1000). Posters will be presented in the Palameeting area, adjacent to where we will have meals. 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 the poster sessions, presenters are asked to stand by their posters as listed in the program schedule each day.

Computer Resources
Wireless internet is available in the Riva Conference Center and instructions for wifi connection will be provided at registration. In addition, three PCs will be located in the foyer of the auditorium (room 1000) and available to access the internet or modify presentations. Keep in mind Italy has 127/220V 50 Hz voltage (http://www.travel-images.com/electric-plugs.html) and you should check to determine if you will need a converter and/or plug adaptor to charge your electronics.

Hospitality
After the evening sessions there will be informal socializing and poster gazing in the Palameeting area. Refreshments will be provided each night until 1 AM. On Saturday evening the informal socializing in the Palameeting area will include music.

Steering Committee
Please share your suggestions and comments about the meeting with the 2010 Steering Committee:
Jane Dorweiler, Chair (jane.dorweiler@marquette.edu)
Mike Muszynski, Co-Chair (mgmuszyn@iastate.edu)
Erik Vollbrecht, Co-Chair (vollbrec@iastate.edu)
Giuseppe Gavazzi (giuseppe.gavazzi@unimi.it)
Karen Koch (kekoch@ufl.edu)
Robert Bensen (robert.bensen@syngenta.com)
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Kathy Newton, treasurer, ex officio (newtonk@missouri.edu)
Mary Schaeffer, abstract coordinator, ex officio (Mary.Schaeffer@ars.usda.gov)
Carson Andorf, abstract coordinator, ex officio (Carson.Andorf@ars.usda.gov)

Acknowledgements
This meeting would not have been possible without the dedicated effort of many people. Firstly, we thank our local hosts Gabriella Consonni, Giuseppe Gavazzi, Mario Motto and Roberto Tuberosa who have been invaluable in helping to navigate the numerous organizational and funding matters associate with our first conference in Europe. Secondly, we thank Carson Andorf and Mary Schaeffer for their tremendous efforts in organizing and assembling the conference program, Julio Rodriguez for his design of the poster and new MGC logo and Enrico Coen for the program cover art. We also thank Angela Freemeyer and her team at the University of Missouri Conference Center for helping to organize the conference, handling registration and dealing with a multitude of other issues. Special thanks are also extended to the staff of Riva del Garda Conference Center and Rivatour – Alberta Costa, Graziella Zucchelli, Silvia Bordignon and Elisabetta Ballardi – for their help in organizing this conference. Additional thanks go to Kathy Newton for her help in managing the finances for this meeting and Bob Bensen, Mark Cigan, Roberto Tuberosa and Jane Dorweiler for their efforts to secure funding in support of graduate student attendance at this meeting. Finally, many thanks go to Marty Sachs for his wisdom in all things related to the Maize Meeting.

Schedule of Events

Thursday, March 18

3:00 PM – 6:00 PM	**REGISTRATION / POSTER HANGING**
5:30 PM – 7:00 PM	**DINNER**
7:00 PM – 7:20 PM	**WELCOME AND ANNOUNCEMENTS**
7:20 PM – 8:30 PM	**SESSION 1** Chair: Jane Dorweiler
7:20 PM	**Dedication of a special *Maydica* issue**
7:40 PM	**Marianne Bänziger, CIMMYT (Plenary 1)** *Stress tolerant maize for the developing world - Challenges and prospects.*
9:00 PM – 12:00 AM	**INFORMAL POSTER VIEWING**
12:00 AM – 1:00 AM	**HOSPITALITY**

Friday, March 19

8:15 AM – 8:30 AM	**ANNOUNCEMENTS**
8:30 AM – 10:10 AM	**SESSION 2 – Functional and Quantitative Genomics** Chair: Giuseppe Gavazzi
8:30 AM	**Nathan Springer, University of Minnesota (T1)** *Combined analysis of genomic structural variation and gene expression variation between maize and teosinte populations*
8:50 AM	**Joost van Heerwaarden, University of California, Davis (T2)** *A second look at the geographic origin of maize*
9:10 AM	**Michael McMullen, USDA-ARS / University of Missouri (T3)** *Genome diversity, recombination and selection in maize: Evidence for the Hill-Robertson effect on fitness and heterosis*
9:30 AM	**Julie Fievet, UMR Génétique Végétale (T4)** *The metabolic model of the genotype-phenotype relationship reveals the role of epistasis in heterosis*
9:50 AM	**Steve Moose, University of Illinois (T5)** *Genetic Control of Nitrogen Utilization in Maize*
10:15 AM	**BREAK**
10:45 AM – 12:25 PM	**SESSION 3 – Development and Cell Biology** Chair: Paula McSteen
10:45 AM	**Michael Muszynski, Iowa State University (T6)** *The proximal-distal pattern of maize leaf growth is altered by mutations in a cytokinin signaling protein*
11:05 AM	**John Humphries, University of California San Diego (T7)** *The role of a receptor-like protein and ROP GTPases in the polarization of asymmetric cell divisions in the maize leaf epidermis*
11:25 AM	**Mihaela-Luiza Márton, University of Regensburg (T8)** *Pollen Tube Guidance by ZmEA1 Signalling in Maize and Arabidopsis*
11:45 AM	**Thomas Hartwig, Purdue University (T9)** *na1 (nana plant1) encodes a brassinosteroid biosynthetic enzyme that controls in addition to plant height, sex determination and tillering in maize.*
12:05 PM	**Kimberly Phillips, The Pennsylvania State University (T10)** *vanishing tassel2 (vt2) encodes an auxin biosynthesis gene required for vegetative and inflorescence development in maize*
12:30 PM – 1:30 PM	**LUNCH**
1:30 PM – 5:00 PM	**POSTER SESSION 1** *Presenters should be at odd numbered posters from 1:30 PM to 3:00 PM.* *Presenters should be at even numbered posters from 3:00 PM to 4:30 PM.* *Refreshments will be available from 3:30 PM to 5:00 PM.*

Friday, March 19

5:00 PM – 6:00 PM	**SESSION 4 – GENOME UPDATES** Chair: Mark Cigan
5:00 PM	**Doreen Ware, USDA-ARS / Cold Spring Harbor Laboratory (T11)** *Update of Maize B73 Sequencing Project: New Resources to Study Maize Evolution*
5:12 PM	**Fusheng Wei, University of Arizona (T12)** *Maize B73 RefGen_v2, a significant improvement from v1*
5:24 PM	**Pierre Montalent, UMR Génétique Végétale (T13)** *EuGène-maize: a web site for maize gene prediction*
5:36 PM	**Thomas Brutnell, Boyce Thompson Institute (T14)** *Characterization of the maize leaf transcriptome through ultra high-throughput sequencing*
5:48 PM	**Carolyn Lawrence, USDA-ARS / Iowa State University (T15)** *Sequence resources at MaizeGDB with emphasis on POPcorn: a PrOject Portal for corn*
6:20 PM – 8:00 PM	**SESSION 5 – PLENARY TALKS** Chair: Michael Muszynski
6:20 PM	**Anne Sylvester, University of Wyoming (Plenary 2)** *Balancing Cell Division and Expansion during Maize Leaf Development*
7:10 PM	**Michele Morgante, Università di Udine (Plenary 3)** *The pan-genome concept in plants: origin, structure and function of the dispensable genome*
8:00 PM – 9:00 PM	**DINNER**
9:30 PM – 12:00 AM	**INFORMAL POSTER VIEWING & HOSPITALITY**
12:00 AM – 3:00 AM	**HOSPITALITY**

Saturday, March 20

8:30 AM – 10:10 AM	**SESSION 6 – BIOCHEMICAL GENETICS** Chair: Uta Paszkowski
8:30 AM	**Mario Motto, Unità di Ricerca per la Maiscoltura (T16)** *The Zea mays mutants opaque-2 and opaque-7 reveal extensive changes in endosperm metabolism as revealed by protein, amino acid, and transcriptome-wide analyses*
8:50 AM	**Bryan Gibbon, Baylor University (T17)** *Suppression of opaque2 Phenotypes by Altered Starch Granule Structure*
9:10 AM	**Thomas L. Slewinski, The Pennsylvania State University (T18)** *tie-dyed2 regulates carbohydrate export in maize leaves*
9:30 AM	**Belmiro Vilela, CRAG - Centre for Research in Agrogenomics (T19)** *ZmSnRK2.8 is involved in stomatal closure and phosphorylates two maize transcription factors*
9:50 AM	**John Woodward, Cornell University (T20)** *A maize thiamine auxotroph is defective in shoot meristem maintenance*
10:15 AM – 10:45 AM	**BREAK**
10:45 AM – 12:25 PM	**SESSION 7 – Quantitative Genetics and Breeding** Chair: William Tracy
10:45 AM	**Catherine Bermudez Kandianis, University of Illinois (T21)** *Rare allelic variation in Zea mays crtRB1 Increases beta-carotene in maize grain*
11:05 AM	**Sherry Flint-Garcia, USDA-ARS / University of Missouri (T22)** *Genetic architecture of maize kernel quality in the Nested Association Mapping (NAM) population*
11:25 AM	**Marc Albertsen, Pioneer Hi-Bred Int., Inc (T23)** *From basic reproductive biology to novel hybrid seed production*
11:45 AM	**Jode Edwards, USDA-ARS / Iowa State University (T24)** *Inheritance of adaptation to high plant density in the Iowa stiff stalk synthetic maize population*
12:05 PM	**Edward Buckler, USDA-ARS / Cornell University (T25)** *Genome wide association study of leaf architecture finds key genes that have contributed to maize improvement over the 20th century*
12:30 PM – 1:30 PM	**LUNCH**

Saturday, March 20

1:30 PM – 5:00 PM **POSTER SESSION 2**

Presenters should be at even numbered posters from 1:30 PM to 3:00 PM.
Presenters should be at odd numbered posters from 3:00 PM to 4:30 PM.
Refreshments will be available from 3:30 PM to 5:00 PM.

5:00 PM – 6:00 PM **Community Discussion led by Maize Genetics Executive Committee**

6:20 PM – 8:00 PM **SESSION 8 – PLENARY TALKS**

Chair: Erik Vollbrecht

Enrico Coen, The John Innes Centre (Plenary 4)
Development of Shape in Plants

Alain Charcosset, INRA-CNRS-UPS-AgroParisTech Station de Végétale (Plenary 5)
Genomics of quantitative traits: insights into maize adaptation to Europe and prospects for marker assisted breeding

8:00 PM – 9:00 PM **DINNER**

9:00 PM – 12:00 AM **INFORMAL POSTER VIEWING**

12:00 AM – 3:00 AM **HOSPITALITY**

Sunday, March 21

9:10 AM – 10:10 AM	**SESSION 9 – TRANSPOSONS AND EPIGENETICS** Chair: Bob Bensen
9:10 AM	**Karl Erhard, University of California, Berkeley (T26)** *Trans-generational action of Pol IV defines a subfunctionalized expression domain of the Pl1-Rhoades allele*
9:30 AM	**Maike Stam, University of Amsterdam (T27)** *b1 paramutation: the heritable transfer of epigenetic information in trans*
9:50 AM	**Damon Lisch, University of California, Berkeley (T28)** *Changes in epigenetic marks at MuDR are associated with phase change*
10:15 AM – 10:45 AM	**BREAK**
10:45 AM – 12:25 AM	**SESSION 10 – EPIGENETICS AND DEVELOPMENT** Chair: Karen Koch
10:45 AM	**Ryan Douglas, Cornell University (T29)** *ragged seedling2 encodes an ARGONAUTE7-like protein required for leaf patterning, but not dorsiventrality, in maize*
11:05 AM	**Manjit Singh, Institut de Recherche pour le Développement (T30)** *An ovule specific ARGONAUTE protein regulates gamete development in maize*
11:25 AM	**George Chuck, Plant Gene Expression Center (T31)** *The maize SPL transcription factor tasselsheath4 regulates bract development and establishment of meristem boundaries*
11:45 AM	**Liliana Costa, University of Oxford (T32)** *Regulation of nutrient transfer cells in the maize endosperm by the imprinted gene Meg1*
12:05 PM	**David Skibbe, Stanford University (T33)** *Transcriptome and proteome support for organ-specificity of Ustilago maydis tumor induction in maize*
12:25 PM	**ADJOURNMENT**

Posters

Biochemical Genetics

P1	**Norman Best** <nbbest@purdue.edu>	*A pharmacological approach to isolate brassinosteroid biosynthesis and signaling mutants in maize.*
P2	**Linlin Zheng** <monika.frey@wzw.tum.de>	*Analysis of DIMBOA expression in maize*
P3	**Kristin Chandler** < kchandl@purdue.edu >	*Analysis of Segregation for Orange and Yellow Color and Phenotypic Selection for Carotenoid Content in Maize Kernels*
P4	**John Fernandes** <john.fernandes@stanford.edu>	*Analyzing Picogram Quantities of Maize RNA*
P5	**Thomas L. Slewinski** <tls315@psu.edu>	*Carbohydrate partitioning defective1 is essential for phloem function and plant viability*
P6	**Barbara Wozniak** <Barbara.Wozniak@unil.ch>	*Characterization of variation in mycorrhizae responsiveness in maize*
P7	**Wanchen Li** <aumdyms@sicau.edu.cn>	*Cloning and Characterization of Functional Trehalose-6-phosphate Synthase Gene in Maize*
P8	**Hoshang Rahmati** <hoshang.rahmati@yahoo.com>	*Comparison of yield in two corn hybrid Navid-bakhsh (Ksc700) and commercial hybrid (Ksc704)*
P9	**Ruairidh Sawers** <ruairidh.sawers@unil.ch>	*Disruption of the LOOPHOLE (LPH) MFS-TRANSPORTER Of rice confers resistance to colonization by arbuscular mycorrhizal fungi*
P10	**Yunbi Xu** <y.xu@cgiar.org>	*Development of an integrated SNP linkage map and its use in detection of recombination frequency variation and segregation distortion regions*
P11	**Silvia Fornale** <silvia.fornale@cid.csic.es>	*Effect of downregulation of Cinnamyl Alcohol Dehydrogenase (CAD) on lignin biosynthesis in transgenic maize CAD-RNAi plants.*
P12	**Regina Dick** <Regina.dick@wzw.tum.de>	*Evolution of chemical defense pathways: Benzoxazinoid biosynthesis in Zea mays and Consolida orientalis*
P13	**Yubi Huang** <yubihuang@sohu.com>	*Expression property of ADP-glucose pyrophophosrylase-encoding genes*
P14	**Yonglian zheng** <zhyl@mail.hzau.edu.cn>	*Fine mapping and Cloning of QTL for plant height using Near isogenic Introgression Lines (NILs) of Maize*
P15	**Siva Prasad Kumpatla** <spkumpatla@dow.com>	*Flexible and Cost-Effective SNP Genotyping Platforms for Low to High-Throughput Applications in Maize*
P16	**Jing Yuan** <J.Yuan.2@warwick.ac.uk>	*Identification of members of the MEG1 complex in maize transfer cells*
P17	**Sylvia Morais de Sousa** <smsousa@cnpm.embrapa.br>	*Influence of phosphorus and sucrose in root morphology, biochemistry and physiology*
P18	**Marco Chiapello** <marpello@email.it>	*Towards deciphering plant-fungal dialogues*
P19	**Yonglian Zheng** <zhyl@mail.hzau.edu.cn>	*Revelation on response and molecular mechanism of waterlogging tolerance in maize roots*
P20	**Shu-Yi Yang** <Shu-Yi.Yang@unil.ch>	*Rice phosphate transporters OsPT11 and OsPT13 are essential for arbuscular mycorrhizal symbiosis*
P21	**Lifang Zhang** <zhangl@cshl.edu>	*Studies on waterlogging responsive miRNAs and their transcriptional regulation in maize roots*

P22	**Roberto Pilu** <salvatore.pilu@unimi.it>	*The accumulation of anthocyanins pigment in the kernel is altered by the low phytic acid1-241 maize mutation*
P23	**Robert Baker** <rfb11@psu.edu>	*The search for defective plasmodesmata in the phloem of tie-dyed2 mutant leaves*
P24	**Poster removed**	

Bioinformatics & Computational Biology

P25	**Matthew Hufford** <mbhufford@ucdavis.edu>	*Influence of Cryptic Population Structure on Observed Mating Patterns in the Wild Progenitor of Maize (Zea mays ssp. parviglumis)*
P26	**Nick Lauter** <nick.lauter@ars.usda.gov>	*An integrated expression profiling system for maize*
P27	**Matt Evans** <mmsevans@stanford.edu>	*Analysis of the Maize Gametophytic Transcriptomes*
P28	**David Hessel** <dhessel@iastate.edu>	*COGENFITO: a new module of MaizeGDB to optimize isoline selection in breeding schemes*
P29	**Cecilio Mota** <cmota@conabio.gob.mx>	*The accumulation of anthocyanins pigment in the kernel is altered by the low phytic acid1-241 maize mutation*
P30	**Siva Prasad Kumpatla** <spkumpatla@dow.com>	*Development and Validation of High-throughput Resistance Gene Analog (RGA) SNP assays in Maize*
P31	**James Schnable** <jschnable@berkeley.edu>	*Following tetraploidy in maize, a short deletion mechanism removed genes preferentially from one of the two homeologs*
P32	**Brian Smith-White** <smtwhite@ncbi.nlm.nih.gov>	*Plant Genomic Resources at National Center for Biotechnology Information*
P33	**Carson Andorf** <carson.andorf@gmail.com>	*MaizeGDB: Tools And Resources*
P34	**Mary Schaeffer** <mary.schaeffer@ars.usda.gov>	*MaizeGDB -- The Data*
P35	**Lisa Harper** <ligule@berkeley.edu>	*How to use the new sequence-based functionalities MaizeGDB*
P36	**Ethalinda Cannon** <ekcannon@iastate.edu>	*POPcorn: A PrOject Portal for corn*
P37	**Tanja Pyhajarvi** <tanja.pyhajarvi@oulu.fi>	*Population structure of Zea mays ssp. parviglumis*
P38	**Jon Beck** <jbeck@truman.edu>	*SAMgm: An Information Resource for the Functional Annotation of Maize Genes*
P39	**Mike Freeling** <freeling@berkeley.edu>	*The pan-grass synteny project*
P40	**Matthieu Falque** <falque@moulon.inra.fr>	*Two Types of Meiotic Crossovers Coexist in Maize*
P41	**Gregory Downs** <gdowns@uoguelph.ca>	*Using NAM polymorphism data to identify molecular markers in Corn Belt Dent inbred lines.*

Cytogenetics

P42	**Fangpu Han** <fphan@genetics.ac.cn>	*A functional maize centromere without CentC and CRM sequences*
P43	**Weiwei Jin** <weiweijin@cau.edu.cn>	*Coix centromeres contain evolutionary conserved repetitive DNA sequences*
P44	**Ashley Lough** <anl6d9@mizzou.edu>	*Dynamics of Mitochondrial DNA Insertions into Maize Chromosomes*
P45	**han zhang** <hzhang@plantbio.uga.edu>	*In vivo visualization of chromosome movement using protein tethering*
P46	**Matt Evans** <mmsevans@stanford.edu>	*Mechanisms underlying reduced transmission of B-A chromosome segments from tertiary trisomic stocks*
P47	**Inna Golubovskaya** <innagol@berkeley.edu>	*Meiotic mutants affecting homologous synapsis*
P48	**Kulvinder Gill** <ksgill@wsu.edu>	*Now is this the Ph1 gene?*

Development & Cell Biology

P49	**Lin Li** <lli06@uoguelph.ca>	*Characterizing development...*
P50	**Davide Sosso** <davide.sosso@ens-lyon.fr>	*emb8522 encodes a chloroplast-targeted pentatricopeptide repeat (PPR) protein necessary for maize embryogenesis*
P51	**Clinton Whipple** <whipple@byu.edu>	*sterile tassel silky ear1 (sts1) Regulates Stamen and Lodicule Identity*
P52	**Marina Dermastia** <marina.dermastia@nib.si>	*A cellular study of teosinte Zea mays ssp. parviglumis (Poaceae) caryopsis development showing several processes conserved in maize*
P53	**Philip Becraft** <becraft@iastate.edu>	*A negative regulator of aleurone development functions downstream of dek1*
P54	**Charles Hunter** <ibe@ufl.edu>	*A role in leaf epidermal development for Cellulose Synthase-Like D1*
P55	**Timothy Kelliher** <tkellih1@stanford.edu>	*Anthers in 3-D: cell division, morphology, and fate from primordium to microsporogenesis*
P56	**Rena Shimizu** <rs334@cornell.edu>	*Analyses of WOX3 regulation and candidate target genes involved in mediolateral leaf development*
P57	**Mo Jia** <mo_jia@baylor.edu>	*Analysis of TOR kinase Pathway Gene Expression in Developing Maize Kernels and Opaque Mutants.*
P58	**Paula McSteen** <pcm11@psu.edu>	*Auxin Evo-Devo: Genetic and genomic approaches to understanding the role of auxin in shoot development*
P59	**John Matera** <jtm5m3@mail.missouri.edu>	*Changes in Mitochondrial Genomes Associated with Reversions of S-Type Cytoplasmic Male Sterility in Maize*
P60	**Suzhi Zhang** <suzhi1026@163.com>	*Characterization of the correlation between ZmSERK gene expression and embryogenesis in maize culture*
P61	**Joke Baute** <jobau@psb.vib-ugent.be>	*Cold and drought stress induce different cellular and molecular growth responses in maize leaves*
P62	**Olena Liapustina** <Liapustina@gmail.com>	*Culture of isolated embryo sacs and caryopses as a biotechnological system for the upgrowing of maize zygotic embryos in vitro for genetic transformation procedures*

P63	**Christopher Bozza** <cgb25@cornell.edu>	*Dissecting Homologous Pairing Using the SegII Mutant*
P64	**Andreas Lausser** <andreas.lausser@biologie.uni-regensburg.de>	*Elucidating the Role of the Extracellular Peptide ZmEBP1 for Embryo Patterning*
P65	**Antonino Malgioglio** <antonio.malgioglio@unimi.it>	*Embryos desiccation tolerance in maize viviparous mutants*
P66	**Simon Malcomber** <smalcomb@csulb.edu>	*Evolution of RAMOSA3-like genes in grasses (Poaceae)*
P67	**Daniel Hill** <harkius@uwyo.edu>	*Expression and localization of ZmRAB2A and ZmRAB1A in the developing maize leaf*
P68	**Brent O'Brien** <bob2373@ufl.edu>	*Expression of the Maize Cellulose Synthase (CesA) Gene Family at the Cell-, Protoplast-, and Tissue- Levels.*
P69	**Brent Buckner** <bbuckner@truman.edu>	*Expression of the Maize Chromatin Assembly Factor-1 Genes*
P70	**Thomas Dresselhaus** <thomas.dresselhaus@biologie.uni-regensburg.de>	*Female Gametophyte Cell Identity in Maize is Regulated by diSUMO-Like (ZmDSUL) and EA1-Box Protein ZmEBP1*
P71	**Yanxiang Zhang** <yanxiang.zhang@zmbp.uni-tuebingen.de>	*Functional characterization of RUM1 protein-protein interactions*
P72	**Eric Riedeman** <riedeman@wisc.edu>	*Genetic Background Effects on Phenotypic and Genotypic Expression of Cg1*
P73	**Katie Petsch** <petsch@cshl.edu>	*Genetic analysis of the trans-acting siRNA (ta-siRNA) pathway and adaxial-abaxial patterning in maize*
P74	**Masaharu Suzuki** <masaharu@ufl.edu>	*Genetic network regulating development of embryo and lateral organs by Viviparous8 and Big embryo1*
P75	**Nathalie Bolduc** <nath.bolduc@gmail.com>	*Genome-wide identification of KNOTTED1 targets using ChIP-Seq.*
P76	**Patrice Dubois** <pgd7@cornell.edu>	*Hormonal and temperature regulation of far-red light signaling in the maize seedling.*
P77	**Victor Gonzalez** <gonzalev@uoguelph.ca>	*Insight into density tolerance – is plant to plant variability the key to density tolerance?*
P78	**Poster removed**	
P79	**Alexander Goldshmidt** <goldshmi@cshl.edu>	*Investigation of molecular mechanisms controlling determinacy of the spikelet-pair meristem.*
P80	**Nikolay Manavski** <fbga024@botanik.uni-hamburg.de>	*Isolation of a RNA-binding protein gene from a collection of Mutator-induced seed mutants*
P81	**Laura Morales** <lauramo@ufl.edu>	*Kernel number per ear: A role for sorbitol dehydrogenase*
P82	**Hank Bass** <bass@bio.fsu.edu>	*Maize SUN Domain Proteins; A 5-Member Gene Family Encodes Two Distinct Classes (CCT and PM3 types) of Putative Nuclear Envelope Proteins.*
P83	**Manfred Gahrtz** <manfred.gahrtz@biologie.uni-regensburg.de>	*Maize Stem Parenchyma – Developing Tools for the Engineering of a Potential Storage Tissue*
P84	**Jerome Martin** <jemar@psb.vib-ugent.be>	*Meristem determinacy: ear versus leaf*

P85	**Christine Majer** <christine.majer@zmbp.uni-tuebingen.de>	*Molecular interactions of the LOB domain transcription factor RTCS which is involved in shoot-borne root formation of maize*
P86	**Rachel Wang** <rachelcjw@berkeley.edu>	*Multiple archesporial cells 1 (mac1) is required for cell fate determination during anther development*
P87	**Montserrat Pages** <montse.pages@cid.csic.es>	*New functions for maize CK2 b subunits*
P88	**Ljudmilla Timofejeva** <ljuda_timofejeva@yahoo.com>	*Novel maize mutants impaired in cell differentiation during anther development*
P89	**Michael Pautler** <pautler@cshl.edu>	*Phenotypic characterization and mapping of a fasciated ear mutant.*
P90	**Liza Conrad** <ljconrad@ucdavis.edu>	*Polycomb Genes Controlling Endosperm Development in Rice*
P91	**Dorothee Stoeckle** <stoeckle@uni-hohenheim.de>	*Possibilities to increase the doubling rates in haploid maize seedlings while reducing their mortality*
P92	**Josefine Nestler** <Josefine.Nestler@zmbp.uni-tuebingen.de>	*Proteomic and genetic dissection of root hair formation in maize (Zea mays)*
P93	**Caroline Marcon** <caroline.marcon@zmbp.uni-tuebingen.de>	*Proteomic dissection of heterosis manifestation in developing maize embryos*
P94	**Paolo Sabelli** <psabelli@ag.arizona.edu>	*Regulation and role of the CDK/RBR/E2F pathway in maize transformation and endosperm development*
P95	**Vanessa Vernoud** <vanessa.vernoud@ens-lyon.fr>	*Regulation of the HD-ZIP IV transcription factor ZmOCL1 by a small RNA*
P96	**Yunbi Xu** <y.xu@cgiar.org>	*Revisiting the hetero-fertilization phenomenon in maize using molecular markers*
P97	**Marcela Dotto** <dotto@cshl.edu>	*Role of Lxm1 in leaf patterning and development*
P98	**Katharina R. Haentzschel** <katharina.haentzschel@uni-hohenheim.de>	*Some thoughts on the efficiency of doubled haploid maize production in vivo*
P99	**Viktoriya Coneva** <vconeva@uoguelph.ca>	*Source leaves of the maize indeterminate1 flowering time mutant have elevated carbohydrate levels without a concomitant increase in photosynthetic rate.*
P100	**Priscilla Sofia Manzotti** <priscilla.manzotti@unimi.it>	*Strategies for the isolation of the Shootmeristemless (sml) gene in maize*
P101	**Chih-hua Tsou** <chtsou@gate.sinica.edu.tw>	*Study of maize male floral anthesis by cryo-SEM*
P102	**Damiano Gabotti** <damiano.gabotti@unimi.it>	*The empty PERICARP4 gene is required in post-germinative maize development*
P103	**Tomaz Rijavec** <tomaz.rijavec@nib.si>	*The majority of maize caryopsis cytokinins accumulate in the pedicel*
P104	**Andrea Gallavotti** <agallavotti@ucsd.edu>	*The BARREN STALK FASTIGIATE1 AT-hook transcription factor is required for ear formation*
P105	**Cristian Forestan** <cristian.forestan@unipd.it>	*The PIN-FORMED family of auxin efflux carriers in maize*
P106	**Tatjana Satarova** <satarova2008@yandex.ru>	*The Effect of Auxin Combinations on Callusogenesis of Corn Inbred Lines*
P107	**Cristian Forestan** <cristian.forestan@unipd.it>	*The auxin efflux inhibitor NPA causes aberrant embryo and endosperm development, PIN1 miss-expression and altered auxin accumulation patterns in maize*

P108 **Guenter Theissen** <guenter.theissen@uni-jena.de>
The pod corn phenotype is caused by the ectopic expression of an STMADS11-like MADS-box gene in the ear of maize

P109 **Alessandra Lanubile** <alessandra.lanubile@unicatt.it>
The role of auxins in the development of defective endosperm-18 (de18) mutant mutant of maize: regulation of cell division and endoreduplication

P110 **Kimberly Phillips** <kap262@psu.edu>
Three tasselless (tls) loci are essential for vegetative and reproductive development in maize

P111 **Elizabeth Takacs** <emt32@cornell.edu>
Toward an understanding of the function of DISCOLORED1 (DSC1) in maize kernel development

P112 **David Skibbe** <skibbe@stanford.edu>
Transcriptome and proteome support for organ-specificity of Ustilago maydis tumor induction in maize

P113 **Andrea Eveland** <eveland@cshl.edu>
Transcriptome sequencing and expression profiling during maize inflorescence development

P114 **Mieke Van Lijsebettens** <milij@psb.ugent.be>
Transgenic Research in Maize using Gateway Technology

P115 **Axel Hinze** <fbia037@botanik.uni-hamburg.de>
Transgenic plants producing non-transgenic pollen – A new confinement method for maize?

P116 **Gregorio Hueros** <gregorio.hueros@uah.es>
You'll never walk alone: ZmMRP-1 is not the only Myb related transfer cell specific transcription factor

P117 **Frank Harmon** <fharmon@berkeley.edu>
ZCN8 is likely a central integrator of flowering time signals in maize leaves

P118 **Andrea Skirpan** <als152@psu.edu>
*barren stalk*3112 is a new maize mutant essential for tassel and ear development*

P119 **Peter Bommert** <bommert@cshl.edu>
compact plant2 (ct2) encodes the α-subunit of a heterotrimeric GTPase in maize and is required for the regulation of meristem size

P120 **Young Koung Lee** <leeyk@cshl.edu>
fasciated ear3: mutant characterization and map based cloning in maize.

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P121 **Jong-Jin Han** <han@cshl.edu>
Identification of Mutator Insertion Sites by sequencing the MTM population

P122 **Prem Chourey** <pschourey@ifas.ufl.edu>
A trancriptome profile of basal endosperm transfer cell layer (BETL) in W22 developing seeds.

P123 **Mingliang Xu** <mxu@cau.edu.cn>
A Major QTL for Resistance to Fusarium Stalk Rot in Maize

P124 **Guoying Wang** <gywang@caas.net.cn>
A putative maize zinc-finger protein gene, ZmAN13, may participate in abiotic stress responses

P125 **Mark Cigan** <MARK.CIGAN@PIONEER.COM>
A survey of transcriptional silencing of promoters by pIRs in maize and rice

P126 **Karina van der Linde** <lindek@mpi-marburg.mpg.de>
A virus induced gene silencing system in maize and its application to characterize maize genes involved in the Ustilago maydis/ Zea mays interaction

P127 **Chuanxiao Xie** <cxxie@caas.net.cn>
Allelic Diversity of Two Cytosolic Glutamine Synthtase Family Members Associated with Low Nitrogen Tolerance in Maize

P128 **Shibin Gao** <shibingao@gmail.com>
Analysis of Gene responsible for low phosphorus stress in maize

P129	**Ashraf El-kereamy** <aelkerea@uoguelph.ca>	*Approaches for studying nitrogen use efficiency in maize and rice plants*
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P131	**Michel Zivy** <zivy@moulon.inra.fr>	*Cellular functions affected by water deficit in maize growing leaves : a proteomics approach*
P132	**Yunjun Liu** <yunjunliu@gmail.com>	*Characterization of the antioxidant enzyme genes of glutathione-ascorbate redox cycle in Zea mays*
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P134	**Qing Li** <cauliqing@163.com>	*Dissection of Genetic Basis for Kernel Tocopherols Content in Maize*
P135	**Federico Martin** <fmartin@ufl.edu>	*Distributed Simple Sequence Repeat (SSR) markers for efficient mapping from maize public mutagenesis populations*
P136	**Caroline Gutjahr** <caroline.gutjahr@unil.ch>	*Divergent transcriptional responses of rice root types to arbuscular mycorrhizal colonization.*
P137	**Gaganpreet Sidhu** <gks27@cornell.edu>	*Evolution of meiotic recombination genes in maize and teosinte*
P138	**Thais Figueira** <trsilva@unicamp.br>	*Evolutionary relationship between thhe prolamins of maize, sorghum and sugarcane.*
P139	**Steven Eichten** <eicht021@umn.edu>	*Extensive Copy Number Variation Among Maize Lines*
P140	**Anne Sylvester** <annesyl@uwyo.edu>	*Fluorescent protein tagged maize lines for cell biology and functional genomics*
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P143	**Theresa Musket** <muskett@missouri.edu>	*Genetic Variability for Root Architecture in Maize*
P144	**Rajandeep Sekhon** <rsekhon@glbrc.wisc.edu>	*Genome-wide atlas of gene transcription through maize development from germinating seed to maturing seed*
P145	**Jeff Glaubitz** <jcg233@cornell.edu>	*Genotyping by sequencing in maize*
P146	**Zhiming Zhang** <zhangzm1979@yahoo.com.cn>	*Identification of genes differentially expressed in maize (Zea mays L.) during Rhizoctonia Solani Kühn infection by suppression subtractive hybridization*
P147	**Ana Butron** <abutron@mbg.cesga.es>	*Identification of genes related to germination by screening variability within inbreds*
P148	**Ruth Swanson-Wagner** <swan0589@umn.edu>	*Inter- and Intra-Specific Gene Expression Diversity in Maize*
P149	**Paulo Arruda** <parruda@unicamp.br>	*Is the 19KD α-prolamin gene family in maize and sugarcane a product of a convergent evolution?*
P150	**Erik Vollbrecht** <vollbrec@iastate.edu>	*Large-scale genome mutagenesis with the transposable element Dissociation (Ds)*
P151	**Jon Duvick** <jduvick@iastate.edu>	*Maize Community Annotation Project to Improve Gene Structures*

P152 **Nick Carpita** <carpita@purdue.edu>
Maize Translational Genomics for Improvement of Energy Grasses

P153 **Nina Zellerhoff** <nina.zellerhoff@uni-koeln.de>
Maize ionomics - elemental-fingerprinting towards identification of markers with predictive value for high mycorrhizal nutrient uptake efficiency

P154 **Maureen McCann** <mmccann@purdue.edu>
Maximizing carbon and energy efficiencies of biofuels production

P155 **Alessandra Lanubile** <alessandra.lanubile@unicatt.it>
Microarray analysis of differentially expressed Fusarium verticillioides-induced maize genes

P156 **A. Mark Settles** <settles@ufl.edu>
Mutants in dosage-dependent genes affecting seed composition or weight

P157 **Hank Bass** <bass@bio.fsu.edu>
Nucleosome Mapping and Chromatin Structure in Maize, a Novel Platform for Genome Response Assays.

P158 **Gunther Doehlemann** <doehlemann@mpi-marburg.mpg.de>
Organ specificity in the biotrophic interaction of maize and Ustilago maydis

P159 **Gregorio Hueros** <gregorio.hueros@uah.es>
SSH-Mu-cDNA, Suppression Subtraction hybridisation as an efficient approach for the isolation of mutant-specific Mu-flanking sequence tags.

P160 **Mihai Miclaus** <mihai@waksman.rutgers.edu>
Several approaches to clone opaque-7, a nutritional quality trait in maize

P161 **Don McCarty** <drm@ufl.edu>
The UniformMu resource for reverse genetics in maize

P162 **Mark Cigan** <MARK.CIGAN@PIONEER.COM>
Transcriptional gene silencing for maize hybrid production

P163 **Anja Paschold** <anja.paschold@zmbp.uni-tuebingen.de>
Transcriptome-wide analysis of the manifestation of heterosis in young seedling roots of maize (Zea mays L.)

P164 **Hilde Nelissen** <hilde.nelissen@psb.vib-ugent.be>
Translational research from Arabidopsis to maize

P165 **Lisa Kanizay** <lkanizay@plantbio.uga.edu>
Uncovering the Sequence and Structure of Maize Abnormal Chromosome 10

P166 **Christine Lucas** <cjlucas@illinois.edu>
Zein-RFP reporter lines as tools to study 22 kD α-zein gene regulation

Education & Outreach

P167 **Anastasia Bodnar** <abodnar@iastate.edu>
Biofortified: An educational resource for plant genetics and genetic engineering

P168 **Anne Sylvester** <annesyl@uwyo.edu>
Maize Cell Genomics as a Springboard for Teaching Genetics and Cell Biology at a Tribal College, Little Big Horn College.

P169 **Robyn Stevens** <stevens@ncga.com>
The National Corn Growers Association: Applying Scientific Knowledge to Ensure Increasing Opportunities for Corn Growers

Quantitative Genetics & Breeding

P170 **Anthony Studer** <studer@wisc.edu>
QTL mapping of a factor upstream of teosinte branched1 (tb1) that effects ear morphology

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Segregation Distortion Results in a Novel Approach to Identify Grain Yield QTLs

P172 **Martin Ganal** <ganal@traitgenetics.de>
A 50K SNP Infinium chip for maize genome analysis

P173 **Etienne Kaszas** <Etienne.Kaszas@SYNGENTA.COM>
A Very High Volume, Fast and Flexible Genotyping Platform for Marker Assisted Breeding

P174 **silvia giuliani** <silgiul27@yahoo.com>
A major QTL on bin 1.06 controls root and agronomic traits in maize across water regimes

P175 **Chris Grainger** <cgrainge@uoguelph.ca>
Artificial Selection and the Genome: "Deep Pedigree" Analysis in an Elite Soybean Cultivar

P176 **Judith Kolkman** <jmk87@cornell.edu>
Association and linkage analysis implicates the Ts2 region in resistance to NLB in maize.

P177 **sophie bouchet** <sophie.bouchet@moulon.inra.fr>
Association genetics investigation of flowering time in a panel of American and European maize inbred lines

P178 **Masanori Yamasaki** <yamasakim@tiger.kobe-u.ac.jp>
Association study in Japanese rice population

P179 **Chiara Lanzanova** <chiara.lanzanova@entecra.it>
BIORES PROJECT: Study of modified b-32 RIP maize proteins in plant protection against pathogens*

P180 **Elena Pestsova** <Elena.Pestsova@uni-duesseldorf.de>
Breeding of chilling tolerant energy maize

P181 **Valeriu Rotarenco** <rotarenco@mail.md>
Breeding procedures at the level of haploid sporophyte in maize

P182 **Ann Stapleton** <stapletona@uncw.edu>
Combining two stresses creates a joint-stress environment that has a different genetic architecture

P183 **Laurence Moreau** <moreau@moulon.inra.fr>
Comparison of QTL detection in intermated and conventional maize recombinant populations derived from a Flint x Dent F1 hybrid.

P184 **Stefan Scholten** <stefanscholten@gmx.net>
Correlation of parental transcriptome and field data for the characterization and prediction of heterosis in maize

P185 **Jason Morales** <jasonmorales@purdue.edu>
Diallel analysis of elite EX-PVP commercial inbreds

P186 **Weiwei Wen** <wenweiwei1982@gmail.com>
Detection on genetic integrity of conserved maize germplasm in genebank using SNP markers

P187 **Leah Viesselmann** <lviesselmann@wisc.edu>
Differential zein protein levels in a su1 population divergently selected for visual endosperm starchiness

P188 **Jason Peiffer** <jap333@cornell.edu>
Dissecting Natural Diversity in Maize Plant Height: GWAS and Fine Mapping of Chr9L QTL

P189 **Carlotta Balconi** <carlotta.balconi@entecra.it>
*Ear secondary traits related to aflatoxin accumulation in commercial maize hybrids under artificial field inoculation.**

P190 **Sofiane MEZMOUK** <sofiane.mezmouk@biogemma.com>
Effect of population structure corrections on the results of association mapping tests in complex maize diversity panels

P191 **Jose Santa-Cruz** <jhsantac@ncsu.edu>
Field Evaluation and Genetic Characterization of a Quantitative Trait Locus conferring Resistance to Southern Leaf Blight

P192	**Susanne Kohls** <susanne.kohls@ipw.agrl.ethz.ch>	*Fine-Mapping of Rf4 , a Major Restorer-of-Fertility Gene for c-Type Cytoplasmic Male Sterility in Maize*
P193	**Ana Belen Monteagudo** <anamonteagudo@ciam.es>	*First steps for breeding galician white corn landraces for traditional-baking bread*
P194	**Elisabetta Frascaroli** <elisabetta.frascaroli@unibo.it>	*From QTL Analysis to Evaluation of Near Isogenic Lines for Heterotic QTL in maize.*
P195	**Peter Balint-Kurti** <peter_balintkurti@ncsu.edu>	*Genetic Analysis and Characterization of Quantitative Resistance to Southern Leaf Blight in Maize*
P196	**Jillian Foerster** <jfoerster@wisc.edu>	*Genetic Architecture of Vegetative Phase Transition in Maize*
P197	**Tatjana Satarova** <satarova2008@yandex.ru>	*Genetic Control of Matroclinal Haploidy in Maize*
P198	**Sara Helland** <sara.helland@pioneer.com>	*Genetic Dissection of Shoot Architecture and Flowering Time Responses to Marginally Differential Photoperiods in Maize*
P199	**Candice Hansey** <cnhansey@wisc.edu>	*Genetic Diversity of a Maize Association Population with Restricted Phenology*
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P201	**Peter Balint-Kurti** <peter_balintkurti@ncsu.edu>	*Genetic dissection of multiple disease resistance in maize*
P202	**Yongtao Yu** <yyt0112@hotmail.com>	*Genetic diversity analysis and heterotic grouping of the sweet corn germplasm in China*
P203	**Pedro Revilla** <previlla@mbg.cesga.es>	*Genetic of the fitness of the sweet corn mutant sugary1*
P204	**Victor Rodriguez** <vmrodriguez@mbg.cesga.es>	*Genetic regulation of the accumulation of pigments at low temperature*
P205	**Claude WELCKER** <claude.welcker@supagro.inra.fr>	*Genetic variability of growth under water deficit in maize: meta-analysis of QTL, modelling and test of allelic effects at consensus QTL*
P206	**Mathew Hooyer** <mhooyer@uoguelph.ca>	*Genotype-by-Environment From a Different Perspective.*
P207	**Jeffrey Jeddeloh** <jeffrey.jeddeloh@roche.com>	*High-resolution mapping and genotyping of maize using long oligonucleotide microarrays*
P208	**Delphine Van Inghelandt** <vaninghe@uni-hohenheim.de>	*How many SSR and SNP markers for population structure and genetic diversity analyses in a commercial maize breeding program?*
P209	**Yongtao Yu** <yyt0112@hotmail.com>	*Identification of QTL for yield-related traits induced by space flight in sweet corn*
P210	**Aida Kebede** <a.kebede@cgiar.org>	*Influence of maternal parentage and season on the in vivo haploid induction rate in tropical maize*
P211	**Marcelo Carena** <marcelo.carena@ndsu.edu>	*Integrating Pre-Breeding with Cultivar Development in Maize*
P212	**Elena Bitocchi** <elena_bitocchi@yahoo.it>	*Introgression from hybrids varieties into landraces populations of maize (Zea mays ssp. mays L.)*
P213	**Yunbi Xu** <y.xu@cgiar.org>	*Joint linkage-linkage disequilibrium mapping increases QTL detection power: a case study of anthesis-silking interval and grain yield for improving drought tolerance in maize*
P214	**Sara Larsson** <sjl65@cornell.edu>	*Lessons From Dwarf8 on the Strengths and Weaknesses of Structured Association Mapping*

P215	**Brian Dilkes** <bdilkes@purdue.edu>	*Maternal control of interploidy lethality*
P216	**Marion Truntzler** <truntzler@moulon.inra.fr>	*Meta-Analysis of QTL for Traits involved in Silage Quality of Maize and Comparison with the Position of Candidate Genes*
P217	**Andreas Hund** <andreas.hund@ipw.agrl.ethz.ch>	*Meta-analysis of QTLs controlling root length of maize*
P218	**Kelly Robbins** <KRRobbins@dow.com>	*Modeling Stalk and Root Lodging as a Zero-Inflated Proces*
P219	**Paul Zurek** <prz@duke.edu>	*Multidimensional analysis of maize root system architecture in vivo.*
P220	**Peter Balint-Kurti** <peter_balintkurti@ncsu.edu>	*Mutant-assisted exploration of natural variation underlying R gene-mediated immunity in maize*
P221	**David Hessel** <dhessel@iastate.edu>	*Native Resistance to Corn Rootworm Beetles: The Path from Germplasm Screening to Allele Deployment*
P222	**Torbert Rocheford** <torbert@purdue.edu>	*Nested Association Mapping for Maize Tassel and Ear Architecture*
P223	**Valeriu Rotarenco** <rotarenco@mail.md>	*New inducers of maternal haploids in maize*
P224	**Qing Chao** <xinqing009@126.com>	*Mapping and Cloning of a major QTL against head smut in maize*
P225	**Kenda Meade** <kameade@iastate.edu>	*Phenotypic analysis of biomass and moisture contents in testcrossed double-haploids of maize*
P226	**Tiffany Jamann** <tmj35@cornell.edu>	*Progress towards the Positional Cloning of Three Northern Leaf Blight Quantitative Disease Resistance Loci*
P227	**Yuling Li** <yuling_li@126.com>	*QTL detection for grain weight and grain filling rate at four stages after pollination and three starch-related enzymes at 30 DAP*
P228	**Yongtao Yu** <yyt0112@hotmail.com>	*QTL mapping for Pericarp Thickness in sweet corn*
P229	**silvia giuliani** <silgiul27@yahoo.com>	*QTL mapping for root characteristics at the seedling stage in maize*
P230	**Rosa Ana Malvar** <rmalvar@mbg.cesga.es>	*QTLs and genes for Mediterranean corn borer resistance*
P231	**Jianhua Wang** <wangjh63@cau.edu.cn>	*QTLs controling the deep-seeding tolerance in maize*
P232	**Laurent Décousset** <laurent.decousset@biogemma.com>	*QTLs for Yield Components in Maize: complexity reduction by Long-Range PCR and preliminary results of association mapping analysis.*
P233	**Claudia Tschirner** <claudia.tietze@uni-duesseldorf.de>	*Research Strategies Towards Improvement of Nitrogen Use Efficiency in Maize*
P234	**Santiago Mideros** <sxm2@cornell.edu>	*Resolving the genetic architecture of resistance to Aspergillus flavus in maize*
P235	**Belén Salleres** <belensalleres@ciam.es>	*Selection of SSR molecular markers to search for QTLs associated with agronomic traits of grain and forrage maize*
P236	**Fabiano Pita** <fpita@dow.com>	*Stability assessment of hybrids in Multi-Environment trials without replicates.*
P237	**Fabiano Pita** <fpita@dow.com>	*Stability assessment of hybrids in Multi-Environment trials without replicates.*

P238	**Oliver Ott** <ooott@ncsu.edu>	*The Assessment of Gametophyte Factors in North Carolina Inbred Lines*
P239	**Ronald Phillips** <phill005@umn.edu>	*Transforming corn from a commodity crop to a higher-energy, multipurpose biofuel crop.*
P240	**Marymar Goncalves Butruille** <Marymar.Butruille@pioneer.com>	*Tropical Rust Molecular Breeding in Corn*
P241	**Cinta Romay** <mcr72@cornell.edu>	*Unraveling a flowering time QTL on chromosome 8*
P242	**Bicheng Yang** <yangbicheng@genomics.org.cn>	*Whole Genome Resequencing for crop improvement*

Transposons & Epigenetics

P243 **Allison Barbaglia** <ambarbag@oakland.edu>	*Accessing the transcriptional activity of Helitron-captured genes of maize*
P244 **Limei He** <limei@waksman.rutgers.edu>	*Estimates of conversion tract lengths in dimorphic heterozygotes*
P245 **Chunguang Du** <duc@mail.montclair.edu>	*Analysis of Ac/Ds transposons in the maize inbred lines B73 and W22*
P246 **Alexandra Zavalishina** <zavalishinaan@info.sgu.ru>	*Cytoplasm-Induced Paramutations In Maize*
P247 **Nathanael Ellis** <nate.ellis8@gmail.com>	*Examining Histone Modifications in the Pericentromere of Maize*
P248 **Vincenzo Rossi** <Vincenzo.Rossi@entecra.it>	*Functional characterization of the nfc102 maize gene*
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P250 **Massimiliano Lauria** <lauria@ibba.cnr.it>	*Identification of natural variation in dna methylation in maize inbred lines using a genome methylation analysis*
P251 **Zhiming Zhang** <zhangzm1979@yahoo.com.cn>	*Identification and prediction of miRNAs from maize seedling roots responsed to lead (Pb) stress using deep sequencing*
P252 **Christy Gault** <cgault@ufl.edu>	*Mapping and phylogenetic analysis of Mu transposon sequences in maize and teosinte inbreds*
P253 **Thomas Peterson** <thomasp@iastate.edu>	*Mechanism and Genetic Impacts of Transposon Ac/Ds-Induced Rearrangements in Maize*
P254 **Jonathan Gent** <gent@plantbio.uga.edu>	*On the Role of RNA in Centromere Chromatin*
P255 **Lyudmila Sidorenko** <lyudmila@ag.arizona.edu>	*Paramutation is regulated differently at distinct loci*
P256 **Angelo Viotti** <viotti@ibba.cnr.it>	*Parental line dependent expression of specific alpha-zein isoforms in maize endosperm depends upon gene methylation state and genotype-specific maternal factors*
P257 **Ann Armenia** <armeniaa@msu.edu>	*Sequence acquisition by Mutator elements in maize*
P258 **Hervé LASSAGNE** <herve.lassagne@biogemma.com>	*The Maize Gene Machine in Biogemma : an overview*
P259 **PoHao Wang** <puw116@psu.edu>	*The maize Unstable factor for orange1 is required for maintenance of histone modification at pericarp color1 gene*
P260 **Jun Huang** <junhuang@waksman.rutgers.edu>	*The spectrum of mutations produced at a locus by the autonomous transposon Ac*
P261 **Sara Castelletti** <sara.castelletti@studio.unibo.it>	*Uncovering the Methylation Dynamics at the Maize Flowering Time Locus Vgt1*

Plenary Talk Abstracts

Plenary 1

Stress tolerant maize for the developing world - Challenges and prospects

(presented by Marianne Banziger <m.banziger@cgiar.org>)
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Increasing demands for the main food staples, climate change, and increasing water, nutrient and land costs give a new urgency to developing and making available stress tolerant crops. This urgency is the greatest in the developing world where investments in research, capacity building and infrastructure development still lag far behind the developed world. The presentation gives an overview of CIMMYT's investment in the development of stress tolerant maize which has recently gained significant leverage through stronger research collaboration with public and private partners, and now extends from native and transgenic trait discovery to large scale application of marker assisted selection approaches tailored to the improvement of highly quantitative traits such as yield under drought and low soil fertility. Many years of CIMMYT research indicate that these traits are highly polygenic, which has implications for the use of transgenics, identification of effects within association mapping studies, and the choice of appropriate marker based breeding strategies. In addition to assessing front line transgenics originating from the private sector for use in particular in Africa, current efforts focus on marker assisted recurrent selection (MARS), which is being implemented in over 40 biparental populations in Africa, Asia, and Latin America. Current MARS populations are selected on an index of 200 to 300 anonymous SNP markers, a density chosen because it is affordable with current genotyping technology. In 2010, pilot projects on the implementation of genomic selection (GS) using much higher marker densities will be initiated on new platforms based on next generation sequencing technologies, and it is expected that by 2011 genotyping costs will have dropped enough to permit their routine application across the CIMMYT maize breeding program and facilitate innovative native gene discovery and allele mining approaches. With that, CIMMYT is among the first public sector breeding programs that integrate cutting edge transgenic and molecular techniques on a large scale for germplasm development and dissemination to the tangible benefit of resource poor farmers.

Funding acknowledgement: Bill & Melinda Gates Foundation, Howard G. Buffett Foundation

Plenary 2

Balancing Cell Division and Expansion during Maize Leaf Development

(presented by Anne Sylvester <annesyl@uwyo.edu>)
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Maize leaves grow by orderly cell division and cell expansion in a developmental gradient. Leaf shape is established early when the cone-shaped primordium, encircling the base of the meristem, elongates upward. Cells are aligned during leaf elongation by oriented cell divisions followed by directional cell expansion. We study how maize leaf cells execute this organized pattern of cell division and expansion in response to growth cues. Understanding this process is important because leaves are the primary transducers of light energy and leaf shape can affect photosynthetic function. Genetic studies show that precise cell positions are not required during early remodeling of the leaf primordium, but it is not yet understood how linear files of expanding cells are established. We use the accessible leaf epidermis as a cellular model because genetic disruptions are distinct: changes in cell shape are easily detected in a field of normally well-aligned epidermal cells. Using this approach, we have identified mutants that cause cells to over-expand in wart-like clusters, often with minimal impact to overall leaf shape. We use these mutants, including *warty1*, *warty2* and others, as tools to dissect control of expansion in both individual cells and across growing tissues. Lesions in genes that encode RAB proteins, small GTPases that function as vesicle transporters, have been identified and these are hypothesized to be important in membrane recycling or in subcellular organization during wall growth. We have also developed fluorescently tagged marker lines that allow direct observation of protein dynamics during normal and abnormal expansion. Some of these fluorescent marker lines, such as those expressing Zm EXPANSIN-RFP, Zm RAB2A-YFP, Zm RAB1A-CFP and Zm TUBULIN-YFP, are informative because they show spatially and temporally controlled localization patterns within and across fields of cells during expansion. We offer this cellular perspective and specific cell biological tools to the research community for all levels of investigation.

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Plenary 3

The pan-genome concept in plants: origin, structure and function of the dispensable genome.

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The comparative sequencing of several plant genomes revealed that transposable elements are
largely responsible for extensive variation in both intergenic and local genic content not only between closely related species but also among individuals within a species. In addition larger structural variants can be detected, similar to the copy number variants identified in the human genome and involving hundreds of Kbp of DNA and tens of genes. A single genome sequence may therefore not reflect the entire genomic complement of a species and prompted us to introduce in plants the concept of the pan-genome, which includes core genomic features common to all individuals and a dispensable genome composed of non-shared DNA elements that can be individual- or population-specific. The pan-genome model has recently been proposed also for the human genome, where, however, the size of the dispensable genome seems to be negligible when compared to that observed in some plant species. We will describe what has been learned so far about the dispensable genome in two species whose genome has been fully sequenced, maize and grapevine. In both species extensive variation among genotypes can be detected as structural variants due either to simple transposable element insertions or to insertions/deletions of large genomic regions. We will describe the size and composition of the dispensable genome as well as discuss possible molecular mechanisms leading to such variation. Uncovering the intriguing nature of the dispensable genome, namely its composition, origin and function, represents a step forward towards an understanding of the processes that generate genetic diversity and phenotypic variation.

Plenary 4

Development of Shape in Plants

(presented by Enrico Coen <enrico.coen@bbsrc.ac.uk>)
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Much progress has been made recently in our understanding of how genes control patterns of cell types or regional identities within an organism during its development. However, the link between this process of patterning and growth or morphogenesis is much less well understood. Bridging this gap requires a quantitative understanding of how genes modify growth of multicellular tissues in 3D space at multiple scales. We have been addressing this problem using a combination of genetic, morphological, computational and imaging approaches in collaboration with Andrew Bangham (University of East Anglia) and Przemyslaw Prusinkiewicz (Calgary). The results provide new insights into how genes interact with patterns of growth at various scales to modify shape. The talk will illustrate how integrating biological and computational methods may lead to a quantitative mechanistic framework for development.

Funding acknowledgement: BBSRC

Plenary 5

Genomics of quantitative traits: insights into maize adaptation to Europe and prospects for marker assisted breeding

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After the Discovery of the new world by Europeans, maize rapidly spread in European regions displaying contrasted climates. Molecular analyzes highlight the major role of early flowering North American ("Northern Flints") introductions, cultivated as such or after hybridizations with tropical origins which formed Europe specific gene pools. Later, with the transfer of the hybrid technology to Europe, European gene pools adapted to cool European climate proved particularly complementary to Northern American materials to create high yielding hybrids adapted to local conditions.
We will then present how statistical meta-analyses of results obtained in diverse linkage mapping populations, involving European materials and including multiparental designs, contribute to the identification of the main regions of the genome involved in flowering time architecture. Combination of linkage based fine mapping and association genetics enables to refine the nature and the allelic series at these QTLs. This will be illustrated by two case studies in the region of vgt1 and for a major photosensitivity QTL located on Chromosome 10.
Finally, we present the application of marker assisted selection following QTL mapping in a multiparental experimental design involving four European inbred lines. Experimental evaluation of plants selected at each generation shows a significant genetic gain through cycles of selection conducted on markers only. Interest of this approach is also supported by simulations, illustrating that strategies addressing a broad diversity and using present high-throughput genotyping technologies should prove highly beneficial to accelerate genetic gain for production and adaptation to environmental constraints.

Funding acknowledgement: French National Research Agency, French Consortium Génoplante, Promaïs, INRA

Short Talk Abstracts

T1

Combined analysis of genomic structural variation and gene expression variation between maize and teosinte populations

(presented by Nathan Springer <springer@umn.edu>)

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Maize is known to be a highly diverse species with high levels of DNA sequence, gene expression and phenotypic variation among different genotypes. Previous studies have identified sub-populations of maize through the use of SNP or SSR molecular markers. Our goal was to identify gene expression variation and genomic structural variation differences among sub-populations of maize as well as between maize and teosinte. A custom long-oligonucleotide microarray was used to interrogate both expression and genomic copy number in 43 diverse maize inbred lines as well as 24 teosinte genotypes. The expression profiling data was used to identify groups of genes that exhibit differential expression between maize and teosinte or among different sub-populations. In addition, it is possible to identify genes with highly conserved expression levels across many genotypes. The comparative genomic hybridization data can be used to identify examples of copy number variation (CNV) and presence-absence variation (PAV) in the genomes of different inbred lines. Even using relatively stringent criteria there are over a thousand genes that are present in B73 but missing in some other lines and several hundred genes with additional copies in some genotypes. The majority of examples of CNV and PAV are present in multiple lines suggesting that these are not recent, novel events. It was possible to identify examples of CNV or PAV that are restricted to certain sub-populations of maize or teosinte. The combination of expression profiling and comparative genomic hybridization provides a detailed view of how gene expression and genome structure have been shaped by artificial selection in maize.

Funding acknowledgement: National Science Foundation (NSF)

T2

A second look at the geographic origin of maize

(presented by Joost van Heerwaarden <jvanheerwaarden@ucdavis.edu>)

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The biological and geographic origin of maize has long been of great interest to crop scientists and archeologists. The last decade has seen important advances in our knowledge of maize domestication. Genetic studies have provided firm evidence that maize was domesticated from Balsas teosinte (*Zea mays* ssp. *parviglumis*), a wild relative that is currently endemic to mid- to lowland regions of central Mexico. An interesting paradox remains however: while Balsas teosinte shows the closest affinity to maize, the most ancestral maize accessions seem to originate from the Mexican highlands, where Balsas teosinte does not currently grow. Thus, although the ecology of maize's wild ancestor points to a lowland origin, genetic data seems to suggest domestication in the highlands. Recent archeological findings of the earliest cultivated maize supports lowland domestication, confirming the need to reconsider the genetic evidence. We use a recently compiled dataset of ~800 SNPs, scored in a large number of georeferenced accessions of both teosinte and maize to take a new look at the geographic origin of maize. We find that ongoing gene flow between maize and its wild relatives meaningfully impacts our inference of the geographic origins of maize. We also show that assignment of an inferred ancestral population to a map of estimated gene frequencies yields different results from the traditional direct comparison of maize to teosinte. We discuss the implications of these results for reconciling ecological, archeological and genetic data on maize origins and potential application of the method to general questions of geographic origin.

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

T3

Genome diversity, recombination and selection in maize: Evidence for the Hill-Robertson effect on fitness and heterosis

(presented by Michael McMullen <mcmullenm>)
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Recent studies on maize inbreds have demonstrated an unprecedented level of genetic variation that includes high levels of SNP and small indel diversity along with varietal differences in transposon, gene fragment and expressed gene content. These differences result from the demographic history of maize which involves an out-crossing mating system, large effective population size, and very deep coalescence. During the genetic characterization of the nested association mapping (NAM) population, a reference design with 26 founders and 5000 RILs, we noted a non-random genomic distribution of residual heterozygosity in the S5 generation RILs. Residual heterozygosity is higher within pericentromeric regions than in the more distal regions of all 10 chromosomes. There is an inverse relationship between residual heterozygosity and recent recombination across the genome ($r^2 = 0.35$), which is much higher than the relationship of residual heterozygosity with either gene density or SNP diversity. The Hill-Robertson effect states that efficiency of selection at multiple loci is reduced by linkage. Therefore, plant breeding progress during inbred development will be decreased in regions of low recombination rate. Our data support this hypothesis. The implications of our results on plant breeding and the genetic basis of heterosis will be discussed.

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

T4

The metabolic model of the genotype-phenotype relationship reveals the role of epistasis in heterosis

(presented by Julie B Fievet <fievet@moulon.inra.fr>)
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Described for more than two centuries, heterosis, or superiority of a hybrid over its parents for quantitative traits, remains poorly understood. The interactions between genes possibly responsible for heterosis are established since many years: dominance (the parents are complementary for the "good" alleles of the genes controlling the trait), overdominance (heterozygosity is an advantage *per se*) and epistasis (interactions between genes involved in the trait). However, there is no general theory of heterosis because the genetic and molecular approaches to this phenomenon usually do not rely on any model of the genotype-phenotype relationship. We chose the flux through a metabolic network as model quantitative trait, and used a generalization of the Kacser & Burns' biochemical model for dominance and epistasis to analyse the genetic consequences of the joint variation of several enzymes of the network. We hypothesized that metabolic heterosis should be observed because the response of the flux towards enzyme activities and/or concentrations follows a multi-dimensional hyperbolic-like relationship. To corroborate this, we simulated the genetic variability of four enzymes of the upstream part of glycolysis by varying *in silico* and *in vitro* the enzyme concentrations. From each distribution of enzyme concentrations (i.e. each "parent"), we computed the flux. Then we "crossed" these parents to get hybrids, and compared the hybrid and parental flux values. Due to the concavity of the flux-enzyme relationship, mid-parent heterosis was observed. More interestingly, best-parent heterosis was observed whenever the distributions of enzyme concentrations were contrasted. The decomposition of the flux value into genetic effects, with the help of a novel multilocus epistasis index, revealed that antagonistic additive-by-additive epistasis effects play the major role in this framework of the genotype-phenotype relationship. This result is consistent with various observations in quantitative and evolutionary genetics, and provides a model unifying the genetic effects underlying heterosis.

T5

Genetic Control of Nitrogen Utilization in Maize

(presented by Steve Moose <smoose@illinois.edu>)
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Increasing the productivity of maize with lower nitrogen (N) inputs will have many positive benefits to world agriculture. Nitrogen utilization is a measure of the plant's ability to produce biomass relative to the amount of accumulated N. The genetic control of N utilization in maize is complex, but reflects a simple physiological switch where acquired N is used either to promote additional growth (biomass) or is conserved for later use by the plant or its progeny. Through functional genomics analysis of N utilization in diverse maize germplasm spanning the last century of maize breeding, we have identified asparagine (Asn) cycling as a key regulatory pathway for N utilization in maize. Changes in allelic distributions and expression among genes in the Asn cycling pathway are associated with three important physiological responses to 20th century selection for increased grain yields and N utilization: 1) higher harvest index (grain biomass/total biomass), 2) reduced grain N (i.e. protein) concentration, and 3) the negative relationship between grain yield in low N soils and magnitude of response to supplemental N fertilizer. RNA expression analysis and metabolite profiling demonstrate that coordinated reciprocal regulation of Asn synthesis and conversion to other amino acids modulates N utilization. Genetic mapping and targeted resequencing studies reveal that functional variation in the expression of Asn cycling genes is programmed by both *cis*-acting promoter variants and factors acting in *trans* on multiple genes within the pathway. Collectively, our results indicate that changes in Asn cycling contributed to past improvements in maize N utilization and also suggest strategies to further enhance N utilization in maize and related cereal or bioenergy crops.

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T6

The proximal-distal pattern of maize leaf growth is altered by mutations in a cytokinin signaling protein

(presented by Michael Muszynski <mgmuszyn@iastate.edu>)
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Maize leaves have distinctive patterns of growth polarized along 3 axes – medial-lateral, abaxial-adaxial and proximal-distal. These patterns are established early in developing, leaf primordia and are essential for normal leaf morphology. Along the proximal-distal axis, the maize leaf consists of four specific tissues: the proximal sheath, the central, wedge-shaped auricle and fringe of epidermal tissue, called the ligule, and the distal blade. The semi-dominant *Hairy Sheath Frayed1 (Hsf1)* mutation disrupts the normal proximal-distal leaf pattern transforming parts of the distal blade into more proximal tissue, such as sheath, auricle and ligule. Ectopic expression of class I *knotted1like homeobox (knox)* genes in developing leaf primordia also disrupts the differentiation of tissues along the proximal-distal leaf axis producing similar phenotypes. How ectopic *knox* expression alters proximal-distal growth is not known, although *knox* genes are known to regulate the accumulation of the plant hormones gibberellic acid and cytokinin. We previously showed that in transgenic plants expressing the *knox* gene *knotted1* in the leaf, treatment with exogenous cytokinin modified proximal-distal development to produce a phenotype resembling the *Hsf1* mutation. This suggested *Hsf1* function, and some element(s) of stereotypical proximal-distal perturbations, may relate to cytokinin signaling. We cloned the gene underlying the *Hsf1* mutation and found it encodes one of the maize cytokinin receptor histidine kinase genes, *ZmHis-protein kinase1 (ZmHK1)*. Mutations in cytokinin receptors had not previously been known to affect leaf pattern specification. Identification of the molecular lesions in three semi-dominant *Hsf1* mutant alleles suggests a mode of action to explain the mutant phenotype. We will present additional results from studies that aim to determine the function of the *hsf1* gene and better understand its role in regulating leaf patterning.

Funding acknowledgement: National Science Foundation (NSF)

T7

The role of a receptor-like protein and ROP GTPases in the polarization of asymmetric cell divisions in the maize leaf epidermis

(presented by John Humphries <jhumphries@ucsd.edu>)
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Polarization of cell division is critical for the generation of cellular diversity in plants, but little is known about the mechanisms governing division asymmetry. In maize, the formation of stomatal complexes involves the polarization of asymmetric subsidiary mother cell (SMC) divisions toward the adjacent guard mother cell (GMC). We found that the maize *pan1* gene promotes the premitotic polarization of SMCs and encodes a leucine-rich repeat receptor-like protein that becomes localized in SMCs at sites of GMC contact. Analysis of the *pan1* mutant phenotype has revealed altered actin localization and depolarization of the nucleus in the SMC, leading to subsidiary cell defects. Although PAN1 has an inactive kinase domain, it is required for the accumulation of a membrane-associated phosphoprotein, suggesting a function for PAN1 in signal transduction. ROP GTPases and an unidentified gene (*pan2*) have also been implicated as part of the PAN1 pathway. While *rop* mutations in maize cause only weak subsidiary cell defects, they have been shown to strongly enhance the *pan1* phenotype. Maize ROP GTPases, like PAN1, accumulate in SMCs at the junction with the GMC, and a physical association with PAN1 has been demonstrated. Our findings implicate PAN1 and ROP GTPases in the transmission of an extrinsic signal that polarizes asymmetric SMC divisions in the maize stomata.

Funding acknowledgement: National Science Foundation (NSF)

T8

Pollen Tube Guidance by ZmEA1 Signalling in Maize and *Arabidopsis*

(presented by Mihaela-Luiza Márton <mihaela.marton@biologie.uni-regensburg.de>)
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During the evolution of flowering plants, their sperm cells have lost mobility and are transported from the stigma to the female gametophyte via the pollen tube to achieve double fertilization. Pollen tube germination, growth and guidance are multi-stage processes largely governed by the maternal sporophytic tissues of the stigma, style and ovule. However, the last phase of the pollen tube path requires extensive cross-talk between both male and female gametophytes, and until recently little was known about the molecules produced by the female gametophyte that are involved in this process. The most recent development in the field will be presented, with a special focus on pollen tube guidance in maize and the role of secreted candidate signalling ligands. *Zea mays EGG APPARATUS1* (*ZmEA1*) was the first gene identified encoding a candidate extracellular ligand involved in micropylar pollen tube attractance controlled by the female gametophyte. *ZmEA1* encodes a polymorphic precursor protein of 94 amino acids that was shown to be secreted to the cell walls of micropylar nucellus cells. Pollen tubes arrived at the micropyle of *ZmEA1* knock-down plants without penetrating the intercellular space of micropylar nucellus cells suggesting a role for ZmEA1 in micropylar pollen tube guidance. Here, we will show that an N-terminal cleaved predicted mature ZmEA1 protein of 49 amino acids is able to directly attract maize pollen tubes *in vitro* at a concentration of <10 µM. Moreover, *Arabidopsis* ovules expressing ZmEA1-GFP fusion protein driven by the synergid cell-specific *Myb98* promoter are capable to attract maize pollen tubes *in vitro*. Current work is focussed on the identification of the mature ZmEA1 peptide and its receptor.

Funding acknowledgement: German Research Foundation (DFG)

T9

Na1 (*nana plant-1*) encodes a brassinosteroid biosynthetic enzyme that controls in addition to plant height, sex determination and tillering in maize.

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Brassinosteroids (BRs) are polyhydroxylated plant steroid hormones, which are found at highest concentrations in reproductive and growing tissues (pollen, immature seeds and young leaves). In plants, BRs are important for cell elongation and normal development, giving them enormous potential in promoting crop yield and biomass (1). Although the importance of brassinosteroids has been recognized in maize, no BR knock-out mutants and their respective genes have been characterized.

Here we report on the cloning and physiological characterization of *na1*, one of the classical maize dwarfing mutations that was first isolated in 1922 (2). Homozygous *na1* plants are characterized as short erect dwarfs that do not respond to treatment with gibberellins. In addition, we found *na1* mutants to have two further characteristics: increased apical dominance and a strong affinity to undergo pistil development in the male inflorescence (*tasselseed*). We have cloned *na1* by transposon tagging and found it to encode a key enzyme of the brassinosteroid biosynthetic pathway. Consistent with this finding *na1* mutants share striking morphological and physiological similarities exhibited by BR dwarf mutants in other species. Furthermore, wild-type maize plants mimic *na1* mutants in every aspect of their phenotype when treated with BR biosynthesis inhibitors. This suggests that BRs control not only plant height but also sex determination and tillering in maize.

1. M. G. Salas Fernandez, P. W. Becraft, Y. Yin and T. Lübbersted (2009): From dwarves to giants? Plant height manipulation for biomass yield. Trends in Plant Science, Vol. 14, 454-461
2. C. B. Hutchison (1922): Cornell Univ. Exp. Syn. Momoir. 60.1419–1473

T10

vanishing tassel2 (*vt2*) encodes an auxin biosynthesis gene required for vegetative and inflorescence development in maize

(presented by Kimberly Phillips <kap262@psu.edu>)
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Auxin biosynthesis, transport, and response are crucial for plant development. Multiple pathways for auxin biosynthesis have been proposed, but until recently few genes functioning in these predicted pathways had been identified in maize.

We have cloned the *vanishing tassel2* (*vt2*) locus of maize using a positional cloning approach. Phylogenetic analyses indicate that *vt2* is co-orthologous to the tryptophan aminotransferase (*TAA*) genes of Arabidopsis, which function in the indole-3-pyruvic acid (IPA) pathway of auxin biosynthesis. Similar to *TAA* mutants, the severity of the *vt2* mutant phenotype is influenced by temperature. However, unlike the *TAA* mutants, a single *vt2* knock-out results in strong vegetative and reproductive defects. *vt2* mutants have severely reduced plant height due to the production of fewer leaves, as well as significant reductions in inflorescence length and spikelet number compared to normal. The severity of the *vt2* phenotype illustrates the importance of auxin synthesized via the IPA pathway in maize.

A similar phenotype is seen in the maize *sparse inflorescence1* (*spi1*) mutant, which was recently cloned and shown to encode a member of the *YUCCA* gene family functioning in the tryptamine auxin biosynthesis pathway. Both *vt2* and *spi1* show very localized patterns of expression, indicating that local auxin biosynthesis plays a critical role in maize development. In addition, the strong single knock-out phenotypes of *vt2* and *spi1* have provided the first opportunity in plants to observe the effects of simultaneously eliminating function of two important auxin biosynthesis pathways. *vt2; spi1* double mutants show an additive interaction, suggesting that the two genes function independently. In addition, the synergistic interaction between *vt2* or *spi1* and the auxin transport mutant *barren inflorescence2* (*bif2*) has revealed that auxin synthesized by these pathways must be transported for proper vegetative and reproductive development. Therefore, auxin transport and auxin biosynthesis have overlapping roles in maize development.

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

T11

Update of Maize B73 Sequencing Project: New Resources to Study Maize Evolution.

(presented by Doreen Ware <ware@cshl.edu>)
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We will describe new resources available at the project website (www.maizesequence.org), plans for refinement of the assembly and annotation, and application of these data that provide new insights into the origin and evolution of maize. A wealth of new transcript data being applied to the reference sequence is expected to yield a step-change in the quality of current annotations and fill gaps where genes were previously missed. A comprehensive comparative genomics resource incorporates maize and other sequenced grasses and eudicots within a phylogenetic framework, allowing one to trace the evolutionary history of genes and identify lineage-specific expansions and contractions of gene families. We will also describe a reconstruction of the two maize subgenomes dating to the tetraploidy event, patterns of genome stabilization, and evidence of rapid evolution that distinguishes maize from other grass genomes.
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T12

Maize B73 RefGen_v2, a significant improvement from v1

(presented by Fusheng Wei <fushengw@ag.arizona.edu>)
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Recently we published the maize genome (i.e. B73 RefGen_v1) which was first released on March 20, 2009. Subsequent to this release, we made a number of additional improvements to the genome sequence, especially with respect to ordering and orienting sequence contigs within BACs. These Improvements included: a) incorporation ~2,000 BAC/fosmid clones, b) ordering and orienting sequence contigs within BACs using paired-end fosmid sequences, c) ordering and orienting sequence contigs within BACs and across contigs using sorghum-maize synteny, and d) ordering and orienting physical contigs based on the maize optical map. The resultant B73 RefGen_v2 genome sequence is composed of 416 physical contigs that are anchored to the maize genetic map (vs. 405 in v1; 99.2% of the genome), and 19 unanchored contigs (vs. 30 in v1; 6.7 Mb in v2 vs. 17.1 Mb in v1). Among them, 391 contigs are ordered and oriented (vs. 336 contigs in v1; 99% in v2 vs. 94% in v1). In total, over 80% of the sequence contigs were ordered and oriented, whereas in v1, only ~30% were ordered and oriented.
Here we will present a detailed account of our improvements to the B73 reference genome sequence, and will discuss plans for further sequence improvement.

Funding acknowledgement: National Science Foundation (NSF)

T13

EuGène-maize: a web site for maize gene prediction

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A large part of the maize B 73 genome sequence is now available and emerging sequencing technologies will offer cheap and easy ways to sequence areas of interest from many other maize genotypes. One of the steps required to turn these sequences into valuable information is gene content prediction. To date, there is no publicly available gene predictor specifically trained for maize sequences. For this purpose, we have chosen to train the EuGène software which can combine several sources of evidence into a consolidated gene model prediction.

Funding acknowledgement: Agence nationale de la recherche (ANR)

T14

Characterization of the maize leaf transcriptome through ultra high-throughput sequencing

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The recent sequencing of the maize genome has provided new opportunities to explore the maize transcriptome using ultra-high throughput sequencing techniques. Here, we perform the first comprehensive analysis of the maize transcriptome along a developing leaf gradient. Transcripts were examined from four developmental zones and isolated bundle sheath and mesophyll cells of the leaf blade. Over 120 million 32-bp reads were generated and mapped to the maize B73 AGPv1 genome release to elucidate gene structure, define alternative splicing events and quantify transcript abundance. Comparisons with the maize working gene set revealed that 81% and 68% of reads fell within annotated exons in the four developmental zones and laser capture microdisected bundle sheath and mesophyll cells, respectively. A global survey of mRNA splicing from developmental zones detected alternative splicing in the majority of intron-containing genes. We observe that 64% genes are differential expressed between leaf segments from base to tip and 21% of transcripts are differentially expressed between mesophyll and bundle sheath cells isolated at the leaf tip. To visualize these data we have developed several informatics tools including a genome browser display, an electronic fluorescent pictograph browser and a two-cell biochemical pathway viewer. Cluster analysis of the data reveals an extremely dynamic transcriptome and provides new insights into the kinetics of C4 photosynthetic differentiation in the maize leaf.

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T15

Sequence resources at MaizeGDB with emphasis on POPcorn: a PrOject Portal for corn

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MaizeGDB is the maize research community's centralized, long-term repository for genetic and genomic information about the crop plant and model organism *Zea mays* ssp. *mays*. The MaizeGDB team endeavors to meet the needs of the maize research community based on feedback and guidance. Recent work has focused on better integrating sequence information as it becomes available for the B73, Mo17, and Palomero Toluqueño genomes into the MaizeGDB resource. Major endeavors along these lines include the implementation and continued development of the MaizeGDB Genome Browser and the creation of POPcorn, an NSF-funded portal accessible via the MaizeGDB website.

POPcorn aims to enable maize researchers to leverage all available genetic and genomic data irrespective of where the data are stored. At present, it is challenging to locate all of the resources and the different sequence-indexed resources must be searched independently. In addition, when a project's funding period ends, the generated data are often lost because they are not moved to long-term repositories: project sites degrade over time and sometimes disappear entirely. The POPcorn project addresses these challenges by providing: (1) a resource for searching for and browsing maize genomics projects and resources; (2) a single resource that uses Web Services and minimal data warehousing to carry out sequence searches (BLAST) that return BLAST outputs and/or data from all participating projects; and (3) a set of tools that enable collaborators to migrate their data to MaizeGDB, the long-term model organism database for maize genetic and genomic information, at their projects' conclusion. POPcorn is a project ancillary to MaizeGDB and may be accessed online at http://www.maizegdb.org/POPcorn.

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

T16

The *Zea mays* mutants *opaque-2* and *opaque-7* reveal extensive changes in endosperm metabolism as revealed by protein, amino acid, and transcriptome-wide analyses

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The changes in storage reserve accumulation during maize grain maturation are well established; however, the key molecular determinants controlling carbon flux to the grain and the partitioning of carbon to starch and protein are more elusive. The *Opaque-2 (O2)* gene, one of the best-characterized plant transcription factors, is a good example of the integration of carbohydrate amino acids and storage protein metabolism in the maize endosperm development. Evidence also indicates that the *Opaque-7 (O7)* plays a role in affecting endosperm metabolism. The focus of this study was to assess the changes induced by the *o2* and *o7* mutations on maize endosperm metabolism, by evaluating protein, amino acid composition, and transcriptome profiling, and to investigate the functional interplay between these two genes in single and double endosperm mutations.
We show that the overall amino acid compositions of the mutants analyzed appeared similar. Each mutant had a high Lys and reduced Glx and Leu content with respect to wild type. Gene expression profiling, based on a Unigene set composed of 7,250 ESTs, allowed us to identify a series of mutant related up-regulated (17.1%) and down-regulated (3.2%) transcripts. Several differentially expressed ESTs homologous to gene encoding enzymes involved in amino acid synthesis, carbon metabolism (TCA cycle and glycolysis), in storage protein and starch metabolism, in gene transcription and translation processes, in signal transduction, and in protein, fatty acid, and lipid synthesis were identified. Our analyses demonstrate that the mutants investigated are pleiotropic and play a critical role in several endosperm metabolic processes. Pleiotropic effects were less evident in the *o7* mutant, but severe in the *o2* and *o2o7* backgrounds, with large changes in gene expression patterns, affecting a broad range of endosperm-expressed genes involved in several metabolic pathways.

T17

Suppression of *opaque2* Phenotypes by Altered Starch Granule Structure.

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We discovered that an important feature of the vitreous endosperm in Quality Protein Maize (QPM) was alteration of the fine structure of starch granules. Specifically, QPM starch had reduced amylopectin branch length and crystallinity. This results in inter-granule adhesions not observed in wild type or *opaque2* endosperm, and these structures appear to restore kernel hardness in QPM. We want to understand the changes in starch biosynthetic activities in QPM that lead to altered starch granule structure; and to understand how the altered fine structure of the starch changes its association with endosperm proteins to promote vitreous endosperm formation. There are four starch synthesis genes that have unique alleles in isogenic lines of *opaque2* maize that differ in endosperm modification, suggesting that these genes are involved in the suppression of the opaque phenotype. We have also discovered that cytosolic endosperm proteins are able to permeate the starch granules in QPM as the kernels mature. This property can be studied in vitro by incubating with fluorescently labeled dextrans, which shows that the QPM starch granules have a significantly larger size exclusion limit. In future work we will compare the proteins associated with wild type and QPM starch granules. These studies will enable us to understand what contribution granule-granule and granule-protein interactions play in formation of vitreous endosperm in QPM, and provide insight into future improvement of maize kernel quality.

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T18

Tie-dyed2 regulates carbohydrate export in maize leaves

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In maize, sucrose is synthesized in the leaf mesophyll cells. For long-distance transport, sucrose diffuses toward the vascular tissue though abundant plasmodesmata, where it is transferred into the cell wall adjacent to the phloem tissue. Sucrose is loaded into the companion cells and transferred into the sieve elements though plasmodesmata. To better understand the genes that regulate this process, we have isolated and characterized a mutant defective in carbon export from leaves. The tie-dyed2 mutant forms non-clonal yellow and green regions in leaves, where yellow tissue hyperaccumulates carbohydrates, yet green tissue is comparable to wild type. To gain insight into the function of Tie-dyed2, we cloned the gene. Tie-dyed2 encodes a highly conserved enzyme that functions in carbohydrate polymer synthesis essential for many developmental and regulatory processes in plant cells. Phenotypic and physiological studies suggest that tie-dyed2 mutants are defective in symplastic transport of carbohydrates from the companion cells to the sieve elements in the yellow tissue. 14C-sucrose labeling studies show that green tissue is able to normally load and transport sucrose. In contrast, yellow tissue is unable to properly load sucrose into the phloem. However, sucrose is able to be transported through the veins of the yellow sectors. Based on phenotypic observations, the identity of the gene and the developmental stability of the phenotype, we hypothesize that Tie-dyed2 functions in plasmodesmata development at the companion cell-sieve element interface. Gene function, allele analysis and possible roles for Tie-dyed2 in plasmodesmata development will be discussed.

Funding acknowledgement: United States Department of Agriculture (USDA)

T19

ZmSnRK2.8 is involved in stomatal closure and phosphorylates two maize transcription factors

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Plants grow in a dynamic environment that frequently imposes constraints on growth, development and productivity. Abscisic acid (ABA) plays a major role in regulating several developmental and physiological processes, such as seed maturation and germination, and mediating the responses of vegetative tissues to osmotic stress. ABA levels increase in late embryo development shortly before the onset of desiccation and in vegetative tissues under water-deficit stress conditions. ABA triggers both stomata closure and water-deficit tolerance responses by regulating changes in the activity of ion channels and changes in gene expression. Protein kinases are key components for mediating cellular responses, including responses to osmotic changes. Many kinases have been described to be involved in ABA and/or osmotic signalling affecting stomata function and/or gene expression. We have isolated a maize SnRK2 kinase that is constitutive, rapidly activated by ABA and osmotic stress in vegetative tissues and that is capable of complementing the *Arabidopsis ost1-2* mutant, which is defective in the regulation of transpiration upon water stress. Using a yeast two hybrid system we have identified two new types of transcription factors that are potential substrates for this enzyme (ZmbHLH and ZmSNAC1). Transcription factors are interesting study candidates to unravel the molecular mechanisms of SnRK2 kinases since they control cellular adaptation by regulating gene expression and represent important tools for biotechnological crop improvement. We are currently characterizing them and their interaction with SnRK2.

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T20

A maize thiamine auxotroph is defective in shoot meristem maintenance

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Plants can undergo organogenesis throughout their life cycle via the perpetuation of stem cell pools called shoot meristems (SMs). SM maintenance requires the coordinated equilibrium between stem cell division and differentiation, and is feedback-regulated by integrated networks of gene expression, hormonal signaling, and metabolite sensing. Phosphate derivatives of thiamine are cofactors in a variety of carbohydrate metabolic pathways, including the citric acid cycle, glycolysis, and the pentose phosphate pathway. Here we show that recessive mutations in the maize *thiamine biosynthetic1-2* gene (*thi1-2*) condition defects in SM maintenance. Plants homozygous for the *thi1-2 (blk)* mutation show a progressive reduction in SM size, resulting in premature shoot termination. Molecular markers for stem cell maintenance (including *knotted1*, *Zmwuschel1-2*, and *abphyl1*) and organ initiation (including *Zm*PIN~GFP and *narrow sheath1*) reveal that these meristematic functions are progressively compromised in mutant plants, especially in inflorescence and floral SMs. Positional cloning of the *thi1-2 (blk)* mutation identified a predicted missense mutation in a highly-conserved amino acid encoded by the maize *thi1-2* gene located on chromosome 3L. Consistent with chromosome dosage studies suggesting that *thi1-2 (blk)* is a null allele, biochemical analyses confirm that the non-mutant THI1-2 enzyme co-purifies with the substrate whereas the mutant enzyme does not. A nearly identical paralog of *thi1-2*, named *thi1-1*, is located on chromosome 8. Although both paralogs are expressed ubiquitously, transcript accumulation of *thi1-2* is down-regulated by brassinosteroids and is 2-225 fold more abundant than *thi1-1* in vegetative and inflorescence SMs. Heterologous expression studies reveal that THI1-2 is targeted to chloroplasts, providing additional evidence for the synthesis of thiamine in plastids. All thi1-2 (blk) mutant phenotypes are rescued by exogenous thiamine supplementation. These data reveal that the inhibition of thiamine accumulation blocks the proliferative growth of stem cell populations in the maize shoot, and provide additional evidence for the integral role of carbohydrate metabolism and signaling during meristem development.

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T21

Rare Allelic Variation in *Zea mays crtRB1* Increases Beta-carotene in Maize Grain

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With extensive diversity in carotenoid concentration and composition, maize has great potential to effectively and sustainably supply dietary Vitamin A to nutritionally deficient communities worldwide. ProVitamin A carotenoids, including beta-carotene, are quantitatively inherited in maize. Using an association mapping approach, three polymorphisms within *Zea mays beta-carotene hydroxylase 1 (crtRB1)* were found to account for maximally 32% of the variation in endosperm beta-carotene concentration by modifying beta-carotene conversion to downstream carotenoid products. The most rare alleles for the 5'TE, InDel4 and 3'TE polymorphisms were associated with a 4.1, 3.3 and 2.3 fold difference in beta-carotene, respectively. Alteration of carotenoid profiles through *crtRB1* may encompass multiple levels of regulation, as observed by tissue-specific differential expression among *crtRB1* allelic variants and distinct differences in biochemical specificity of *crtRB1* allozymes. *crtRB1* genetic effects were found to be directionally consistent across all evaluated segregating populations and led to increases of 1.1-3.1 fold (0.5-2.8 ug g-1) in homozygotes with favorable allelic variation over that of unfavorable homozygotes. Total carotenoid concentrations were inversely proportional to gains in beta-carotene associated with favorable *crtRB1* haplotypes leading to maximal total carotenoid decreases of 23% per *crtRB1* copy, and suggesting that this gene could be involved in pathway-wide regulation of carotenoid accumulation. Introgression of selected *crtRB1* alleles into several synthetic populations of tropical origin and with high endosperm total carotenoid concentrations was achieved using an allele-specific marker assisted selection (MAS) approach, and led to the development of several families with the top two entries producing 19.8 and 21.1 ug g-1 beta-carotene in greenhouse trials; results from 2009 field replicated trials will be reported. Coupled with selective usage of allelic variation at other carotenoid biosynthesis genes such as *lycopene epsilon cyclase (lcyE)*, natural *crtRB1* variation promises to effectively achieve nutritional targets that, to date, have not been achieved by conventional breeding.

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T22

Genetic Architecture of Maize Kernel Quality in the Nested Association Mapping (NAM) Population

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Many studies have been conducted to identify genes (quantitative trait loci; QTL) underlying kernel quality traits. However, these studies were limited to analyzing two parents at once, often resulting in low resolution mapping of QTL. The maize nested association mapping (NAM) population is a reference design with 26 founders and 5000 RILs. The NAM design simultaneously exploits the strengths of both linkage analysis and association mapping, and integrates natural diversity and genomics technologies. Seed from seven locations of NAM was analyzed by near infra-red (NIR) spectroscopy, and BLUP estimates were obtained for kernel starch, protein, and oil content. Heritability for the three traits ranged from 0.83-0.86. Joint stepwise regression (SAS Proc GLMSelect) was used to fit a family main effect & markers nested within families. Preliminary results indicate that NAM kernel quality is controlled by 46-49 QTL for each trait, explaining 71-79% of the phenotypic variation. Principal component analysis appears to be useful in the identification of candidate genes for QTL shared between traits. Many QTL overlap with regions identified in previous QTL studies, and we are investigating several candidate genes using association analysis and/or fine mapping approaches. The identification of kernel quality genes and their interaction with the environment will enable better manipulation of maize for food, feed, fuel, and industrial purposes.

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

T23

From Basic Reproductive Biology to Novel Hybrid Seed Production

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Reproductive biology research has a long history at Pioneer Hi-Bred, not only with the first cloning of a male fertility gene in maize, but also with the identification of many genetic components that are required for controlling the expression of male fertility genes. One of the results from this research has been the development of a novel genetic system to produce maize hybrids, designated as SPT. This system uses a naturally occurring recessive mutation in a sporophytic gene required for male fertility to create female parent lines that are male sterile when the mutant allele is made homozygous. Fertility restoration is accomplished in the F1 plants by fertilization with pollen from any male carrying a wild-type copy of the male-fertility gene. The key to the system, however, is the development of reliable, cost-effective method to propagate pure populations of male-sterile homozygous recessive lines during female parent seed increase that do not compromise the ability of the lines to tightly maintain male sterility during hybrid production. A system has been developed that uses a unique genetically modified maintainer line to meet the challenge of propagating pure populations of male-sterile female parent lines which cannot be produced by standard self-fertilization techniques. The genetics and theory underlying the SPT system and its use in hybrid seed production will be discussed.

T24

Inheritance of Adaptation to High Plant Density in the Iowa Stiff Stalk Synthetic Maize Population

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Modern maize productivity is largely due to the ability of modern maize to grow and reproduce at high plant density. Specific morphological characters such as upright leaves and reduced anthesis-silking interval have contributed to the ability of maize plants to maintain high levels of productivity in dense stands. Despite a working physiological understanding of contributing physiological and morphological phenotypes, less is known about the genetics of plant-density response in maize. A generation means analysis was performed using Iowa Stiff Stalk Synthetic maize populations in which the original unselected population was compared to advanced cycles of selection and crosses were made to population- and inbred-testers. The cross between the most advanced cycle and the original population showed heterosis for grain yield at all plant densities, but the plant density response for grain yield strongly resembled the original unadapted population, suggesting the adapted phenotypic response in the advance cycle was recessively inherited. Upright leaves were found to have a recessive inheritance pattern in several crosses. These results suggested that phenotypes contributing to adaptation to high plant density in maize may be associated with recessive alleles, unlike the large number of heterotic alleles for which dominant alleles are phenotypically superior. A large mapping study is being developed to map QTL for phenotypes contributing to plant-density response in these populations.

Funding acknowledgement: United States Department of Agriculture (USDA)

T25

Genome wide association study of leaf architecture finds key genes that have contributed to maize improvement over the 20th century

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U.S. maize grain yield has undergone an eight fold increase in the past 80 years, with about half of this increase attributed to genetics. A key aspect of this increase is higher plant densities, which are brought about by changes in leaf architecture that can more efficiently capture light into leaf canopy at high density. We have analyzed leaf architecture traits (leaf length, leaf width and upper leaf angle) using the maize nested association mapping (NAM) population, which integrates the advantages of linkage analysis and association mapping. Additionally, we have developed novel genome wide association (GWA) mapping approaches using the millions of SNPs now being discovered in maize to obtain gene level resolution. By NAM, we found that the genetic architecture of leaf traits is dominated by small effects, with little epistasis or pleiotropy among the leaf traits. In the GWA analysis, we found significant support for several hundred SNPs across the genome (0.05% of tested SNPs) for each trait. In general, the associations were clustered near the linkage peaks. An analysis of candidate genes suggests that known mutants are very good candidates for some leaf architecture QTL. Evaluations of selected candidate genes in a maize association panel support these associations. While there are still limitations with marker density, resolution of available recombination, allele frequency, and model fitting, GWAS with near gene level resolution are becoming a reality for complex traits of maize. Overall, our results suggest that genomic selection based breeding models should be very successful at design of these traits.

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T26

Trans-generational action of Pol IV defines a subfunctionalized expression domain of the *Pl1-Rhoades* allele.

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Plants use distinct subunits of Pol II-related RNA polymerase complexes (Pol IV and Pol V) to repress the expression of transposons and other repetitive sequences in their genomes. In maize, the largest subunit of Pol IV is required for paramutation interactions between *Pl1-Rhoades* (*Pl1-Rh*) alleles of the *purple plant1* (*pl1*) locus [1], for proper sex determination of the male inflorescence, and for abaxial leaf identity [2,3]. Here we describe experimental results indicating that Pol IV defines tissue-specific expression patterns of *Pl1-Rh* in a *trans*-generational fashion. Genetic and molecular data show that *Pl1-Rh* gains novel expression in the endosperm aleurone layer following transmission from a Pol IV mutant. Successive transmissions of *Pl1-Rh* through Pol IV mutants progressively enhances aleurone pigmentation to a condition indistinguishable from that conferred by dominant *C1* alleles of the *pl1* paralog *colored aleurone1* (*c1*). This conditioned regulatory state of *Pl1-Rh* is meiotically heritable even following restoration of wild-type (WT) Pol IV function by outcrossing. Pedigree analyses indicate that the aleurone pigmentation conferred by such conditioned *Pl1-Rh* alleles is dependent upon neither dosage nor parent-of-origin-specific inheritance of either the *Pl1-Rh* allele or WT Pol IV. These data indicate that *trans*-generational action of Pol IV defines the tissue-specific expression domains of specific alleles. This particular example at *Pl1-Rh* highlights a plausible epigenetic mechanism for the subfunctionalization of paralogous gene pairs.

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T27

b1 paramutation: the heritable transfer of epigenetic information in trans

(presented by Maike Stam <m.e.stam@uva.nl>)
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We investigate gene regulation in trans. As a modelsystem we study paramutation, a mitotically and meiotically heritable change in gene expression induced by allele interactions in trans. We examine paramutation at b1, a regulatory gene of the maize pigmention pathway. The low expressed B' epiallele imposes its low transcription rate onto the high expressed B-I epiallele in trans. Recent data indicate a role for RNA in paramutation, but also suggest that siRNAs are not sufficient. We hypothesize a role for physical interactions in addition.

Seven tandem repeats, ~100 kb upstream of the b1 coding region, are essential for b1 trans-inactivation and enhanced b1 expression. Enhancement of b1 expression is tissue-specific and associated with H3ac and nucleosome depletion at the B-I repeats. B' is expressed at a low level and its repeats DNA methylated in a tissue-independent manner. The B' repeats do however also show tissue-specific nucleosome eviction, H3K27me2 and H3K9me2, indicating tissue-specific reinforcement of silencing. Our data indicate that DNA methylation is involved in the heritability of the B' state, while H3ac, H3K27me2 and H3K9me2 are involved in tissue-specific regulation of b1.

3C technology identified tissue-specific and expression level-specific physical interactions at b1. Upon tissue-specific activation of b1, the hepta-repeat and b1 promoter physically interact at the high expressed B-I, but also at the low expressed B' locus, indicating a role in the tissue-specific regulation of b1. 3C further indicates a role for previously unidentified regulatory sequences. These sequences display expression level-dependent interactions with the hepta-repeat and b1 promoter region, indicating they are involved in mediating the high B-I expression level.

Mutants affecting paramutation are being used to address cause and effect relationships.

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Funding acknowledgement: Royal Netherlands Academy of Arts and Sciences

T28

Changes in epigenetic marks at MuDR are associated with phase change.

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A key difference between epigenetic and genetic changes is that epigenetic changes are relatively unstable and dynamic. Thus, time is an essential component of epigenetic regulation. In order to explore this issue, we use the MuDR transposon as a model. By making the appropriate crosses, it is possible to trigger heritable epigenetic silencing of MuDR elements using a derivative of that element called Muk. Our analysis reveals a surprisingly complex process that involves two distinct molecular pathways each of which varies depending on the tissue examined. MuDR carries two genes that are convergently transcribed from nearly identical terminal inverted repeat (TIR) sequences. One gene, mudrA appears to be directly targeted by Muk via homologous RNAs. The TIR of this gene is transcriptionally silenced via a canonical pathway that involves cytosine methylation as well as H3K9 and H3K27 dimethylation. In contrast silencing of mudrB is associated with H3K27 trimethylation and H3 deacetylation, but not with increased DNA methylation. We also find that there are dramatic differences in DNA and histone modification between different leaves at different stages in the development of plants carrying MuDR and Muk. Despite the fact that Muk is not expressed in leaves, we find that in juvenile and adult leaves the promoter of mudrA is modified at both the histone and DNA levels in these tissues. However in growing transition leaves the mudrA promoter lacks these modifications, and transcript is easily detectable. In contrast, marks associated with mudrB silencing are not observed at all in the leaves, and only are apparent in the immature ear. By the next generation, epigenetic marks at both mudrA and mudrB are relatively stable and invariant. We suggest that the observed changes at MuDR during Muk-induced silencing may reflect variation in epigenetic pathways as a function of phase changes during plant development.

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T29

ragged seedling2 encodes an ARGONAUTE7-like protein required for leaf patterning, but not dorsiventrality, in maize

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Leaves are produced from the flank of the shoot apical meristem and are dorsiventrally asymmetric from inception. Mutations perturbing dorsiventral cell-fate acquisition can lead to the formation of radial leaves that lack adaxial/abaxial polarity. However, mutations in the maize (*Zea mays*) gene *ragged seedling2* (*rgd2*) condition radial leaves that maintain dorsiventral polarity. Here, we show that *rgd2* encodes an ARGONAUTE7 (AGO7)-like protein required to produce *ta*-siARF, a trans-acting small interfering RNA that targets *auxin response factor3a* (*arf3a*) transcripts for degradation. Previous studies in maize and rice implicated a role for *ta*-siARF during dorsiventral patterning. Although RGD2 function is required for *arf3a* down-regulation via *ta*-siARF biogenesis, *arf3a* transcripts are polarized upstream of RGD2 function. RGD2 function is also necessary to regulate the accumulation and localization of miR390 in maize shoot apices. Similar to the abaxialized maize mutant leafbladeless1 (lbl1), rgd2 mutants exhibit ectopic accumulation of the abaxial identity factor miR166. These data reveal that hyper-accumulation of *arf3a* and miR166 are insufficient to condition abaxialized leaf phenotypes in maize. Finally, transcripts of a maize *ago1* paralogue over-accumulate in lbl1 but not in rgd2 mutants, suggesting that upregulation of *ago1* combined with ectopic accumulation of miR166 contribute to the formation of abaxialized leaves in the lbl1 mutant. A revised model for the role of small RNAs during dorsiventral patterning of maize leaves is discussed.

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T30

An ovule specific ARGONAUTE protein regulates gamete development in maize

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Apomixis is a form of asexual reproduction through seeds. In apomictic development, female reproductive cells bypass meiosis and fertilization, developing by parthenogenesis offspring that are genetically identical to the mother. In a genetic screen for maize mutant plants mimicking aspects of apomictic development, we identified a dominant mutation resulting in functional unreduced female and male gametes. The mutant shows defaults in chromatin condensation and chromosome segregation during meiosis, resulting in unreduced an aneuploid spore development. The mutated locus codes for a member of the ARGONAUTE family of proteins. In both plants and animals, ARGONAUTES are key players of small RNA dependent silencing pathways. Western blots and immunolocalization experiments using a specific antibody show that the protein is mostly present in the somatic tissues of the ovule at the time of meiosis. We further show that its function is necessary for non-CG methylation of centromeric and knob repeat DNA. Maize has a very complex family of AGO proteins, but phylogenetic and expression data suggest that it is functionally related to Arabidopsis AGO9, which also shows a germ-cell specific phenotype. These results suggest that in plants, as in animals, definition of the germ cells is dependent upon transcriptional repression in the precursor cells of the gametes. Interestingly, the locus is completely down-regulated in the close apomictic relative of maize, Tripsacum. This, together with the phenotype of the mutants, suggests a essential role for small RNA silencing pathways in apomictic reproduction.

T31

The maize *SPL* transcription factor *tasselsheath4* regulates bract development and establishment of meristem boundaries

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Plant architecture consists of repeating units called phytomers, each containing an internode, leaf and axillary meristem. Formation of boundaries within the phytomer are necessary to differentiate and separate these three components, otherwise some will grow at the expense of others. The microRNA targeted SPL transcription factor tasselsheath4 (tsh4) plays an essential role in establishing this boundary within the inflorescence. tsh4 mutants display altered phyllotaxy, fewer lateral meristems, and ectopic leaves that grow at the expense of the meristem. Double mutant analyses of tsh4 and several highly branched mutants such as ramosa1-3 and branched silkless demonstrated a requirement for tsh4 in branch meristem initiation and maintenance. TSH4 protein, however, was localized throughout the inflorescence stem and at the base of lateral meristems, but not within the meristem itself. Double labeling of TSH4 with the ramosa2, branched silkless1 and the knotted1 meristem markers confirmed that TSH4 forms a boundary adjacent to all lateral meristems. Indeed, double labeling of MIR156 showed a meristem specific pattern complimentary to TSH4, consistent with tsh4 being negatively regulated by it. Thus, downregulation of TSH4 by a combination of microRNAs and branching pathway genes allows establishment of lateral meristems and repression of leaf initiation, playing a major role in defining meristem versus leaf boundaries.

Funding acknowledgement: Department of Energy (DOE), BARD

T32

Regulation of nutrient transfer cells in the maize endosperm by the imprinted gene Meg1

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Genomic imprinting predominantly occurs in the endosperm of flowering plants and in the mammalian placenta. While the function of many mammalian imprinted genes appear to be associated with the regulation of nutrient flow to the growing embryo, no plant imprinted genes have thus far been attributed such a function. Here we report on the functional characterization of a maize imprinted gene, Meg1, whose expression is confined to the transfer cells of the basal endosperm. We present data indicating that Meg1 is both necessary and sufficient for transfer cell development. Altered levels of Meg1 transcript in the endosperm affects transfer cell development and nutrient allocation, thus resulting in the production of small seeds with reduced endosperm. Further, our data support the view that in plants, as in mammals, imprinted maternal factors are important for regulating the allocation of nutrients between maternal tissues and offspring - thus representing an example of convergent evolution between flowering plants and mammals.

Funding acknowledgement: BBSRC

T33

Transcriptome and proteome support for organ-specificity of *Ustilago maydis* tumor induction in maize

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The biotrophic fungal pathogen *Ustilago maydis* elicits tumor formation by redirecting development of immature vegetative and floral primordia into a tumor pathway. Injections of wild-type (FB1 + FB2 or the solopathogenic SG200 strain) *U. maydis* into the ~2 cm tassel inflorescence through the leaf whorl of 5-week old plants demonstrated that tumors can form on maturing leaves and all floral organs. A sharp demarcation was commonly observed between tumor-forming regions and areas with normal spikelet maturation and pollen shed; this suggests that both *U. maydis* signals and host responses are restricted spatially. Interestingly, male sterile mutants with defects in anther cell wall differentiation (i.e., prior to meiosis) were unable to form anther tumors. The growth potential mutant *Dwarf8* as well as *spi1* and *fea2* failed to produce tassel tumors while the *Knotted1* mutant produced copious, abnormally large leaf and tassel tumors. On the basis of these phenotypic differences we hypothesized that *U. maydis* tumor formation requires organ-specific gene expression by both partners. A two-organism transcriptome profiling platform was used to identify stage-, organ-, and organism-specific gene expression. A majority of the gene expression differences detected showed that both maize and *U. maydis* express genes in an organ-specific pattern. These data provide strong support for our hypothesis and may explain the organ-specific nature of many plant pathogen infections. Whole proteome experiments are being conducted on infected versus mock tissue samples. Mass spectrometry profiles have already identified over 2000 maize proteins per sample, several hundred of which are detected only in infected or mock tissues. This work was supported in part by an SGER grant from the NSF and the Savitsky Endowment.

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Poster Abstracts

P1

A pharmacological approach to isolate brassinosteroid biosynthesis and signaling mutants in maize.

(submitted by Norman Best <nbbest@purdue.edu>)
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Ever growing demands for biofuel production have led to greater needs for increase in crop yield and specifically biomass. Plant height is among the most imperative components of plant architecture to enhance biomass (1). Since their discovery as growth promoting hormones in 1979, brassinosteroids (BRs) have emerged as essential chemical signals in plant development. Especially their important function in cell elongation gives them huge potential for approaches aimed to manipulate plant height. Unfortunately very little is known about BRs in maize. In fact, no BR knock-out mutant has been isolated and characterized in maize.

Brassinazole-resistant1 (*bzr1*), a transcription factor and key component of BR signaling, was isolated in an *Arabidopsis* screens as being resistant to brassinazole, a BR biosynthetic inhibitor (2). We present a similar pharmacological approach to identify mutants in the biosynthesis and signal transduction of BRs in maize. We utilized resources of the Maize Tilling Project at Purdue (3) to screen for mutants that are either hypo- or hyper-sensitive to applications of the BR biosynthetic inhibitor propiconazole (PCZ). We evaluated mesocotyl elongation of 72,000 EMS- mutagenized M2 seedlings grown for 9d in absolute darkness in the presence of PCZ. Three candidate lines, *propiconazole-resistant1-3* (*pzr1-3*) were isolated, with mesocotyls of more than twice the length of inhibited controls. In addition, one hypersensitive line, *red root hypersensitive1* (*rrh1*) had mesocotyls only 30% the length of inhibited controls in the presence of PCZ.

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P2

Analysis of DIMBOA expression in maize

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Benzoxazinoids are defense chemicals primarily found in the grasses. In maize, the benzoxazinoid DIMBOA is of great importance for the control of the European corn borer (ECB). However, since DIMBOA-biosynthesis is restricted to the young plant, the control is limited to the first generation of ECB. While the biosynthesis of DIMBOA is elucidated, nothing is known about the regulation of the pathway. The pathway genes are located within 4 cM on short arm of chromosome 4 forming a biosynthetic cluster comprising 6 genes that represent three different enzyme classes. This peculiar genomic organization may allow a coordinate gene expression via accessibility of the chromosomal region. As a first step to the isolation of regulatory elements of DIMBOA-biosynthesis we screened the NAM core population for lines that differ with respect to “late” DIMBOA expression. A major difference was detected between Mo17 and B73. Furthermore, a pronounced difference in “late” DIMBOA-content was revealed in the parental lines IL-DF and IL-RD of the KWS introgression line population. The IBM population and the IL population are used for QTL mapping.

P3

Analysis of Segregation for Orange and Yellow Color and Phenotypic Selection for Carotenoid Content in Maize Kernels

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Carotenoids are phytopigments that give many fruits, vegetables and grains their yellow and orange color. Carotenoids are very important nutritionally, as sources of pro-vitamin A for the developing world, and as antioxidants important for eye health for both developing and developed country populations. Maize is a good target crop for carotenoid biofortification due to its broad phenotypic variation for this trait. Simple visual selection for darker orange color has been associated with an increase in total carotenoids. We created a synthetic from the inbreds KUI3, KUI11, KUI43, and KUI2007 and the initial cycle of mass selection for darker orange color resulted in 18.81 µg/g for Lutein, Zeaxanthin, and β-carotene combined, in comparison to 11.65 µg/g for the unselected bulk. Segregation analysis of different populations indicates a relatively major locus is associated with orange color. For example, segregation analysis of different F2:3 ears from the cross of CML 297 x CML 300 revealed various color categories and ratios and such as: 1 Orange: 1 Light Orange-Yellow; 2 Orange: 1 Light Orange-Yellow; and non-segregating Orange. In addition, other segregation analyses will be included for various crosses, such as Hy99 x SaPH876 F2. We are complementing this approach with QTL analysis performed on a A619 x SC55 F2:3 population, with three QTL associated with color categories identified on chromosomes 1 and 9. The QTL on chromosome 9, bin 9.07 mapped to a region containing White cap1, a carotenoid degradation gene. QTL analysis will also be performed on NAM populations that have been color scored and genotyped. We hope that our genetic analysis of distinct color categories will facilitate future phenotypic and genotypic selection for total carotenoids.

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P4

Analyzing Picogram Quantities of Maize RNA

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Determining RNA yield and size in small samples can be problematical. Spectrophotometric and more sensitive fluorimetric detection can determine concentration, but cannot report size distribution. Prior to expensive applications like microarrays or RNA-Seq, it is important to judge both quality and quantity. The Agilent 2100 Bioanalyzer® used with the Plant RNA Nano Assay is suitable for testing total RNA samples of 50 - 500 ng/µL; 50 ng is extractable from about 5000 anther cells and is a small aliquot of amplified RNA samples. Until recently, however, the supplied software for automated quality analysis and intersample comparisons (RIN = RNA Integrity Number) used animal ribosomal RNA sizes and hence was unsuitable for plants, because plant cytoplasmic, mitochondrial, and plastid rRNAs are distinctive from animal sizes. A new Plant RIN program works well for more precisely analyzing size distribution and hence the quality of a sample. We have been interested in switching from pooled staged anthers to much smaller samples of single cell types or very immature anthers for transcriptome profiling. As part of a beta test, we analyzed supplied human RNA samples of 10, 50, and 200 ng using the now released Agilent Low Input Amplification kit with excellent performance on 8 x 15K Agilent human 60-mer oligo arrays using the protocol on the poster. We have begun analyzing 10 – 25 ng maize samples on a 4 x 44K Agilent array using this low input kit. In other experiments we designed ten 60-mer oligo probes across 12 genes to explore the hybridization signal intensity as a function of distance from the 3' end, spanning the typical size of amplified, fluorescently labeled RNA samples (200 – 400 b) and extending further 5'. Supported in part by an NSF PGRP grant (PI Walbot, coPI Cande) and by gifts from Agilent Corporation.

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P5

Carbohydrate partitioning defective1 is essential for phloem function and plant viability

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In plants, transport of carbohydrates from photosynthetic source tissues to non-photosynthetic sink tissues takes place in a specialized group of vascular cells called the phloem. To better understand the genetic regulation of this process, we screened mutagenized populations and have identified a class of mutants that are defective in carbohydrate partitioning and phloem transport. Carbohydrate partitioning defective1 (Cpd1) is a unique semi-dominant mutant isolated from this screen. Cpd1 hyperaccumulates carbohydrates in the leaves when heterozygous for the mutation and is seedling lethal when homozygous. Heterozygous mutant plants strongly resemble the previously characterized sut1 mutant of maize, where leaves hyperaccumualte starch, undergo rapid chlorosis and senescence, and have reduced vegetative and reproductive development. Interestingly, homozygous mutant plants do not accumulate carbohydrates in the leaves and die before leaf four emerges. Aniline blue staining revealed that the heterozygous mutant plants accumulate large aggregates within the sieve elements and at the sieve plate interfaces. These aggregates presumably disrupt phloem transport leading to a build up of carbohydrates in the leaves. The severity of the heterozygous phenotype appears to be temperature dependent; high temperatures exacerbate the phenotype and lower temperatures suppress the phenotype. We mapped the gene to the short arm of chromosome 7. No known genes that function in carbohydrate metabolism or transport have been mapped to this region. Candidate genes and possible roles of the Cpd1 gene will be discussed.

Funding acknowledgement: United States Department of Agriculture (USDA)

P6

Characterization of variation in mycorrhizae responsiveness in maize

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Phosphate is an essential nutrient for normal plant growth. Most of the phosphate is not available to plants due to its immobilization in the soil. One of the ways that plants facilitate phosphate uptake is by forming symbioses with arbuscular mycorrhizal fungi (AMF). During this mutualistic relation the fungus improves the nutrition of the plant, notably by increasing phosphate uptake, and in return it receives photosynthate. Plant varieties are known to vary in the extent of symbiotic benefit, suggesting genetic diversity underlying phenotypic variation.

In our study a core maize diversity panel consisting of 25 lines (Flint-Garcia et al., 2005 Plant J. 44, 1054-1064) were examined for their responsiveness to mycorrhizal colonization. Shoot and root dry weight, and total tissue phosphorus content were measured from mycorrhizal and non-mycorrhizal plants to calculate responsiveness to AM colonization. Furthermore the effect of different phosphorus concentrations on the level of colonization and plant performance were quantified. Moreover to unravel the molecular mechanisms determining mycorrhizal responsiveness we characterized the maize Pht1-like transporters. A total of 14 Pht1-like maize genes were identified in the maize genome and their expression characterized, in the presence or absence of mycorrhizal fungi, under different phosphate levels, and in different cultivars that vary in responsiveness to colonization by AMF.

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P7

Cloning and Characterization of Functional Trehalose-6-phosphate Synthase Gene in Maize

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Trehalose is a non-reducing disaccharide of glucose that functions as a compatible solute in the stabilization of biological structures under heat and desiccation stress in bacteria, fungi and some 'resurrection plants'. In the plant kingdom, trehalose is biosynthesized by trehalose-6-phosphate synthase (TPS) and trehalose-6-phosphate phosphatase (TPP). Over-expression of exogenous and endogenous genes encoding TPS and TPP is reported to be effective for improving abiotic stress tolerance in tobacco, potato, tomato, rice and Arabidopsis.

On the basis of bioinformatics prediction, we cloned a fragment containing an open reading frame of 2820 bp from maize, which encodes a protein of 939 amino acids. Phylogenetic analysis showed that this gene belongs to the class I subfamily of the *TPS* gene family. Analysis of conserved domains revealed the presence of a TPS domain and a TPP domain. Yeast complementation with *TPS* and *TPP* mutants demonstrated that this protein has the activity of trehalose-6-phosphate synthase. Semi-quantitative RT-PCR indicated that the expression of this gene is up-regulated in response to both salt and cold stress.

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P8

Comparison of yield in two corn hybrid Navid-bakhsh (Ksc700) and commercial hybrid (Ksc704)

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Corn, a major source of food for both humans and animals, is grown in more countries than any other crop. The importance of corn and its various uses, planning to increase production in order to independency is necessary. In this regard, replacing of high yield variety with traditional and low yield variety has importance role to attain this goal. Thus, an experiment was conducted in three regions of Koohdasht County, that is, central region, Tarhan region and Koohnani region. In this experiment, tow corn hybrid (Ksc 700 and Ksc 704) were compared with each other. In each region tow corn hybrids were sowed by pneumatic seed sower. At physiological maturity the fields were harvested by combine and then seed moisture percentage, yield and net yield were measured. The results showed that, yield of Ksc 700 and Ksc 704 was 13558 and 12772 kg.ha-1, respectively. In conclusion, it observed that, Navid-bakhsh (Ksc 700) hybrid is better than commercial hybrid (Ksc 704) and it is suggested to cultivation in these regions.

Keywords: Corn; Seed yield; Navid-bakhsh hybrid; Single cross hybrid

Funding acknowledgement: United States Department of Agriculture (USDA)

P9

Disruption of the LOOPHOLE (LPH) Mfs-Transporter of rice confers resistance to colonization by arbuscular mycorrhizal fungi.

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The rice gene *LOOPHOLE (LPH)* is a member of the major-facilitator transporter super-family, predicted to encode a protein of 50kD containing 11 trans-membrane helical domains. Although the putative LPH protein exhibits no close orthology with any previously characterized transporter, it contains a domain of unknown function conserved among plant, animal and fungal taxa (DUF895). In a gene-expression profiling experiment, transcripts encoded by *LPH* were found to accumulate to a higher level in the roots of rice plants colonized by an arbuscular mycorrhizal fungus than in control roots. On this basis, reverse genetics screening was used to isolate an insertional mutant allele of *LPH*. Following inoculation with arbuscular mycorrhizal fungi, plants homozygous for the *LPH* insertion allele did not support the development of intra-radical hyphae and fungal structures observed in wild-type plants. In rice, *LPH* is a single copy gene, as is a putative orthologous gene of maize. In contrast, the legume *Medicago truncatula* and the mycorrhizal non-host *Arabidopsis thaliana* both have two, highly similar, *LPH* -like genes. Two *LPH* -like genes were identified also in fission yeast, although, surprisingly, no orthologue is present in budding yeast. To initiate functional characterization of the *LPH*-like/DUF895 proteins, the two *LPH*-like genes of fission yeast were deleted, both individually and as a double-mutant. In addition, a number of reverse screening efforts have been initiated to isolate mutations in the maize *LPH* gene.

Funding acknowledgement: Swiss National Foundation

P10

Development of an integrated SNP linkage map and its use in detection of recombination frequency variation and segregation distortion regions

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Integrated molecular maps and associated genetic information have not been fully exploited for improving our understanding of fundamental issues related to genetics of complex traits and molecular breeding applications. With high-resolution genetic maps, many of these issues can be addressed at a whole genome level. In this study, three recombinant inbred line maize populations with parental genotypes from Africa, Asia and Latin America, i.e., XB (X178 × B73), C5 (Ac7643 × Ac7729/TZSRW) and C6 (CML444 × Malawi), were used to construct an integrated single nucleotide polymorphism (SNP) map, detect variability in genetic recombination frequency and identify segregation distortion regions (SDRs). The integrated map consisted of 1442 molecular markers including 1155 SNPs, spanning 1346 cM. Among 1697 informative SNPs scored from two SNP chips each containing 1536 SNPs, 446 (26%) showed segregation distortion at a=5% across the three populations. A total of 13 independent SDRs were identified, one of which co-located with gametophyte factor ga2 within a region of 2.2 Mbp. A 4.5 fold difference in recombination frequency for the same marker interval in the three different populations was observed. In addition, a 100-fold difference in recombination frequency was observed between different chromosomal regions, ranging from an average of 0.09 cM/Mb for pericentromeric regions to 7.08 cM/Mb for telomeric regions across the three populations. Based on recombination suppression, three bins (1.05, 3.04 and 8.03) were confirmed in this study as centromeric regions. Recombination suppression in non-centromic regions identified 11 regions likely to contain condensed heterochromatin (knobs) in the XB population. This integrated map, along with the genomewide information on recombination frequency variation and segregation distortion generated in this study, will greatly facilitate future genetic studies, map-based cloning and marker-assisted plant breeding.

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P11

Effect of downregulation of Cinnamyl Alcohol Dehydrogenase (CAD) on lignin biosynthesis in transgenic maize CAD-RNAi plants.

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Lignin polymer is mainly composed of three subunits; p-hydroxyphenyl (H), guaiacil (G) and syringyl (S). Lignin increases the strength and stiffness of fibres, improves the efficiency of water transport in the vascular system, and protects plants against pathogen attacks. However, the presence of lignin in the lignocellulosic biomass is a negative value both for digestibility and bioethanol production. At present only some spontaneous maize mutants having brown midrib pigmentation in lignifying tissues have been associated to lignin metabolism; the bm1, defective in CAD, and the bm3 in which the caffeic acid o-methyltransferase gene is disrupted. The bm1 mutant has a 90% reduction of CAD activity in roots but the molecular causes of this mutation are unknown. To gain knowledge on how lignin is produced in maize, we have generated a CAD-RNAi transgenic line showing 80% reduction of CAD activity in roots but transgenic plants do not show the typical brown midrib pigmentation. Transgenic stalks contain smaller but more abundant vascular bundles and a reduction of the sclerenchyma. In transgenic stalks, the total lignin content is not reduced but H and G subunits are increased and S subunits decrease. Moreover, lignin composition is altered by a ten-fold enrichment in cinnamaldehydes. CAD-RNAi midribs show alterations in vascular and mechanical tissues but they display a 6.5% reduction of the total lignin content without affecting its final lignin composition. CAD-RNAi midribs display the induction of several enzymes involved in the synthesis of glucose and have higher levels of free sugars, reduction of starch accumulation and slight reduction in several cell wall polysaccharides. CAD-RNAi midribs display higher degradability than wt plants. On the other hand, free sugars and starch accumulation is not affected in CAD-RNAi stems, but a significant increase in cellulose and xylose was observed and they display higher degradability than wt plants.

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P12

Evolution of chemical defense pathways: Benzoxazinoid biosynthesis in *Zea mays* and *Consolida orientalis*

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Phytoanticipins, reactive metabolites that are present in plants before the challenge by pathogens and pests, are often stored as non-toxic glucosides in the plant cell. Upon pathogen attack phytoanticipins are converted to toxic aglucones by specific glucosidases. Benzoxazinoids are phytoanthicipins that are predominantly found in grasses and sporadically in some species of the dicots (e.g. *Consolida orientalis* (Ranunculaceae), *Lamium galeobdolon* (Lamiaceae)). In maize, DIMBOA (2,4-dihydroxy-7-methoxy-2H-1,4-benzoxazin-3(4H)-one) is the predominant substance. DIMBOA functions as natural pesticide, insecticide and is involved in allelopathy. We compared detoxification (UDP-glucosyltransferase, UGT) and activation (ß-glucosidase, ß-GLU) of benzoxazinoids in grasses and in the dicot *C. orientalis*. Phylogenetic analysis of the respective UGTs and ß-GLUs reveals a polyphyletic origin of the detoxification and activation mechanism in monocots and dicots.

Funding acknowledgement: Deutsche Forschungsgemeinschaft

P13

Expression property of ADP-glucose pyrophophosrylase-encoding genes

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ADPglucose pyrophosphorylase catalyzes the first and limiting step in starch biosynthesis. A comprehensive analysis of AGPase-encoding genes is fundamental for the assessment of the function of this enzyme in source and sink organs. The pattern of expression and sugar or hormone regulation of maize AGPase genes in developing grain and leaf has been studyed in this work. In developing seeds and leaves from 1DAF to 30DAF, the six genes of AGPase exhibited different expression pattern and obvious tissue-specific expression profiling. Agpl1, agpl2 and agps1 both in developing seeds and leaves from the early to the late phase were expressed highly, especially in the leaves. And these isoforms had different response to the induction of sugar or hormone. The 2, 4-D and BA can improve expression level of most genes of the six besides agps1 and agps2, especially the 2, 4-D. Although all of these genes exhibited a inhibited response to ABA alone, the 7-fold to 56-fold increase in transcripts for three large and two small subunit genes could be observed in the treatment with ABA and Sucrose together. The accumulation rate of starch significantly increased from 13DAF, but rapidly decreased as the grain filling proceeded. The AGPase activity exhibited an 'S' model during the whole development, which took on a trend of rectilinear rise from 10DAF to 16DAF. The results presented that the composition of AGPase in maize was tissue and developmental stage-specific to be suited to the particular metabolic demands of a given tissue, a development stage or a specific condition. And we provided some novel evidences that confirmed a possible working model in starch biosynthesis that Glc-1-P or Glc-6-P may be tansported into the plastidial in the early and late stage of grain filling.

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P14

Fine mapping and Cloning of QTL for plant height using Near isogenic Introgression Lines (NILs) of Maize

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Near isogenic Introgression Lines (NILs) derived from backcrossing are useful for QTL fine mapping and cloning. A total of 97 link-up introgression lines were developed by BC-MAS with Zong 3 as the recurrent parent, HB522 as donor. A F2 population was developed by crossing a line containing chromosome segment (bnlg1647-bnlg1447) with Zong3. The plant height QTL were mapped in a chromosomal interval flanking ND51and ND35 using 199 individuals in 2007. Ten new SSR markers were developed in the segment, and then the QTL was finely mapped in a 0.5 cM interval using a more than 3,000 individuals segregation population in 2008 and 2009, respectively. Both ND71 and ND88 were located on two overlapping BAC and about 65 kb apart. Ten ORFs in the region were predicted, of which, eight were predicted to encode retrotransposon protein, two encode functional protein, specifically GA3β -hydroxylase. Expression of GA3β -hydroxylase transcript (GA3ox2) was detected to be higher in IL than that in Zong3, implicated that the gene maybe a potential candidate for the plant height. The association mapping and genetic transformation for the candidate gene are on the way.

In 0.5 cM candidate region, crossovered individuals were selected by MAS to develop 12 overlapping ILs. Phenotype of these overlapping ILs evidenced the genetic effect of the candidate region for plant height. Subsequencely, the candidate gene GA3ox2 was cloned, bioinformatics revealed the gene consist of three exon, encode 1149 bp cDNA, and 382 amino acids. Sequence variation between Zong3 and HB522 mainly is found in promoter and untranslation region of GA3ox2.

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P15

Flexible and Cost-Effective SNP Genotyping Platforms for Low to High-Throughput Applications in Maize

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The use of single nucleotide polymorphism (SNP) markers for marker-assisted selection (MAS) in several crops is on the rise due to large scale SNP discovery efforts as well as the availability of efficient and high-throughput (HTP) genotyping platforms. Although several SNP detection technologies are available in the market, the choice of a platform depends on the purpose and scope of a given project. We have been using Illumina's GoldenGate (GG) assays for rapid genotyping and validation of SNP markers. While this permits HTP screening of a large number of SNPs for development as well as some application projects, it is not economical for other projects that require interrogation of only a small subset of SNPs on a small, medium or large number of samples. Thus, there is a need for inexpensive but robust and flexible genotyping systems that would allow the implementation of projects such as gene-specific SNP assay development, in silico SNP validation, marker saturation of the loci of interest, MAS projects using small number of SNPs etc. The Competitive Allele Specific PCR (KASPar) (KBiosciences, Hoddesdon, Hertfordshire, UK) and TaqMan® OpenArray® Genotyping (Applied Biosystems, Foster City, California, USA) technologies are very attractive and promising for this type of projects. The KASPar assay uses an allele specific oligo extension and fluorescence resonance energy transfer (FRET) for signal generation. Studies conducted by us demonstrated high conversion rate of GG SNPs to KASPar assays and also proved to be very cost effective for the genotyping and validation of small subset of SNP markers. The TaqMan® OpenArray® system offers a flexible format of 16 to 256 assays per OpenArray plate with the potential to generate 400,000 datapoints/day and is suitable for projects where large sample sets need to be interrogated with small core set of SNPs. Results obtained and the utility of these platforms for different SNP applications will be presented.

P16

Identification of members of the MEG1 complex in maize transfer cells

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In maize (Zea mays), the endosperm differentiates a range of tissues that assume distinct roles within the developing seed. At the base of the endosperm, epithelial cells differentiate to form the basal endosperm transfer cell layer (BETL). These transfer cells typically contain secondary wall ingrowths and a plasma membrane complex purported to enhance plasma membrane transport capacity. Many BETL-specific genes have been described in maize. Amongst these, we have evidence for an imprinted gene -maternally expressed gene1 (meg1)- which encodes a small cystein rich peptide with a role in the differentiation of the BETL (unpublished data). In order to establish the regulatory protein network involved in formation of the BETL, we performed immunoprecipitation (IP) experiments to identify potential MEG1 interacting protein partners. Here we describe our findings from IP experiments using a peptide antibody raised against MEG1, and a biotin labeled MEG1 peptide. Of those MEG1-interacting candidates, we identified many proteins involved in the synthesis of energy (such as proton ATPase and vacuolar ATP synthase) as well as some novel transmembrane proteins.

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P17

Influence of phosphorus and sucrose in root morphology, biochemistry and physiology

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Phosphorus is one of the most limiting nutrients for crops in the world and the least efficiently used by plants. To increase the absorption capacity of Pi, plants need to adapt the biochemistry, physiology and morphology of their root system. An alternative to solve this problem is to explore the genetic diversity available and breed more Pi-absorption efficient cultivars in combination with soil management practices. Previously, we evaluated maize genotypes for their Pi uptake and use efficiency by verifying grain yield under contrasting levels of phosphorus. In this study, two contrasting genotypes were used to perform a detailed morphological parameters characterization of the root system of plants grown in nutrient solution. We have standardized the growth conditions in nutrient solution (time course and Pi concentration), as well as the parameters to be assessed. To help test the hypothesis that Pi and sucrose availability have a significance on root growth, we tested three different sucrose concentrations (0, 50 and 150 mM) in low and high Pi conditions (2.5 and 250 uM) on two maize contrasting lines. Concentration of sugars (sucrose, glucose and fructose), Pi, K, Ca, Mg and S were measured from maize roots and shoots, as well as the expression of genes involved in sugar metabolism, Pi response and root morphology. We showed that sucrose influenced maize development, modifying not only genetic and biochemical profiles, but also root morphology. Plants grown on sucrose showed a smaller number of fine roots that had higher sugar content, and the genes involved on these processes were also affected. The shoots from sucrose grown plants presented higher Pi and lower K and Ca content. We showed with this study that low Pi and no sucrose at 12 days after treatment are the most feasible conditions to distinguish maize contrasting genotypes for Pi use efficiency. Some root characteristics that are associated with P acquisition can be used for early screening of more efficient plants. This information is essential to accelerate the breeding process and to support advanced studies in molecular biology and physiology, culminating on the in the development of maize efficient cultivars that use less fertilizers.

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P18

Towards deciphering plant-fungal dialogues

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The arbuscular mycorrhiza (AM) association results from a successful interaction between the majority of terrestrial plants and fungal symbiotic partners in the Glomeromycota. Host plants supply photosynthates to their AM fungal partners and, in return, AM fungi enhance the phosphate and nitrogen nutrition of their hosts. This bi-directional nutrient exchange is believed to take place at intracellular fungal structures, called the arbuscules, that form within the root cortical cells. Development of fungal arbuscules induces polarization of the colonized cell and is accompanied by the synthesis of an extensive periarbuscular membrane. This membrane remains in continuum with the peripheral plasma membrane and governs the molecular dialogue between fungus and plant. While there has been much progress in understanding signaling during the early phase of AM symbiosis development, the regulation of symbiosome development, and nutrient transport are largely unknown.

Proteomics is likely to be the best methodology to unravel the molecular components of the plant-fungus interactions. In a novel proteomics approach we aim at determining the molecular constituents of the periarbuscular membrane and the peripheral plasma membrane sub-domains of the polarized arbusculated cells.

P19

Revelation on response and molecular mechanism of waterlogging tolerance in maize roots

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Waterlogging is one of the primary stress elements to maize in southwest china during spring season. A F2 population was derived from a cross between waterlogging tolerance inbred "HZ32" and a sensitive one "K12 ". Several major QTLs determining waterlogging tolerance were mapped to chromosomes4 and 9. Secondary QTLs influencing tolerance were also located on chromosomes 1, 2, 3, 6, 7 and 10 in two years (2008.2009). Differentially expressed genes have been identified from tolerant inbred "HZ32" and sensitive one "Mo17". cDNA microarray was used for studying the early stage in response to waterlogging. Another global study of transcriptional profile was carried out through maize 47K oligo microarray over the response process subjected to waterlogging. The results have been suggested that waterlogging is more complex than expected and it involves a broad spectrum of genes. It is proposed that the response process can be conceptually divided into two stages, defense in early stage and rescue/adaption in late stage. There are two important candidate genes, annotated as transcriptional factors. One is Zm-brlz containing basic region leucin zipper domain and a DNA binding domain and verificated by transient expression fused with GFP in onion cells. A CAPS molecular marker was developed based on Zm-BLRZ and was located at the peak of a QTL on Chr.9 through mapping of F2 population mentioned above. LD analysis based on the sequencing of full length of Zm-blrz in natural population has revealed several SNPs in the promoter or 3'-UTR are significantly related with some key traits involving in tolerance of waterlogging.

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P20

Rice phosphate transporters OsPT11 and OsPT13 are essential for arbuscular mycorrhizal symbiosis

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Many plants obtain phosphate form the soil via the symbiosis with arbuscular mycorrhizal (AM) fungi. In rice we found that two phosphate transporters genes, *OsPT11* and *OsPT13*, are specifically expressed in roots colonized by AM fungi (1, 2). While OsPT11 belongs to a group of orthologous AM-specific phosphate transporters reported from a number of plant hosts of AM fungi, no homolog for OsPT13 has been reported from dicotyledonous plants. *OsPT11* is strongly expressed in roots colonized by phylogenetically distant *Gigaspora rosea* and *Glomus intraradices*. In contrast, induction of *OsPT13* is weaker in both symbioses but specificity of expression is comparable to *OsPT11*.

We aim at understanding how these two transporters contribute to symbiotic phosphate uptake of rice plants colonized by different AM fungi and what their relevance is for the formation of the AM symbiosis. OsPT11 and OsPT13 can complement the yeast phosphate uptake mutant (PAM2) phenotype, confirming that they encode phosphate translocators. Insertional mutant alleles, perturbing gene expression have been identified for both genes from mutants collection. Fungal development of *Glomus intraradices* was significantly reduced in both mutants with *ospt11* displaying a stronger phenotype compared to the *ospt13*. Interestingly, arbuscule morphology remained wild type. Inoculation of both mutants with *Gigaspora rosea* also resulted in a significant reduction of total root length colonization although the effect was less than in the *Glomus intraradices* symbiosis pronounced, indicating that the interaction with *Glomus intraradices* is more dependent on an intact symbiotic phosphate uptake machinery. In summary, these results suggest that both phosphate transporters are necessary for wild type colonization by the respective fungus, but not for the development of arbuscules.

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P21

Studies on waterlogging responsive miRNAs and their transcriptional regulation in maize roots

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Waterlogging is a critical factor that limits maize production in tropical and subtropical regions. Excessive rainfall in low-lying areas inhibits germination and severely damages seedling growth, leading to yield losses of up to 25-30%. The primary effect of water submergence is an inadequate supply of oxygen to roots causing many physiological, biochemical and molecular responses that progress over different stages of stress. Our work seeks to characterize the transcriptional regulatory gene networks (TRNs) associated with the waterlogging. MicroRNA genes play an important role in plant responses to biotic and abiotic stress. In order to characterize miRNA's involvement in the response to waterlogging stress we conducted small RNA profiling experiments, focusing on the 21-22bp component representing mature miRNAs. Our initial results demonstrate rapid changes in the small RNA expression profiles under waterlogging stress conditions. To further this study, we have used Q-PCR to verify the expression changes of miRNAs and their predicted targets. In addition we have verified the predicted targets of these miRNAs using cleavage assays. Our work suggests that miRNA-mediated regulation plays an important role in waterlogging stress responses.

P22

The accumulation of anthocyanins pigment in the kernel is altered by the Low Phytic Acid1-241 maize mutation.

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Phytic acid (InsP6) is the most plentiful form of phosphate in cereals kernel. Phytic acid is accumulated in the seed, in particular in the scutellum, as a mixed phytate salt of several cations such as potassium, iron, zinc, magnesium, etc. This molecule is degraded by phytase activity during seed germination releasing free phosphate, *myo*-inositol and cations, for seedling growth. Monagastric animals are not able to digest phytate salts that furthermore exhibit an anti-nutritional activity in the feed. For these reasons several breeding programs are developing cereals with lower level of phytic acid compared to traditional cultivar. *Low phytic acid* mutants don't modify the total amount of seed P while reduced phytic acid content associated to a proportionally higher level of free phosphate. Transposon mutagenesis experiments conducted by Shi *et al.* in 2007 demonstrated that *lpa1* gene codified for a Multidrug-Associated-Protein (MRP) named *ZmMRP4* (EF586878).

MRP proteins are transmembrane transporters involved in several functions such as organic ions transport, xenobiotic detoxification, oxidative stress tolerance and transpiration control. In previous studies several mutations have been isolated in this locus causing a reduction of phytic acid content. In particular *lpa1-241* mutation causes a reduction until 90% of phytic acid associated to strong pleiotropic effects on the whole plant. In this work we found that *lpa1-241* line, is able to alter the accumulation of anthocyanins in kernel tissues, in genotypes carrying all the genes for anthocyanins biosynthesis. In this work we shown that *lpa1-241* mutation enhances the accumulation of anthocyanins in the kernel conferring a blue colour of the scutellum in the *lpa1-241* strongest mutant. Furthermore here we presented genetic, physiological, hystological and molecular data supporting the hypothesis that the change of anthocyanins colour is due by a defect in their transportation in the vacuole, causing the accumulation of these molecules in the cytosol.

P23

The search for defective plasmodesmata in the phloem of tie-dyed2 mutant leaves

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The genetic control of carbon movement from photosynthetic (PS) cells into the vein for long-distance transport is poorly understood. Plants employ both symplastic and apoplastic routes to transfer fixed carbon from PS cells into the vein. Symplastic transport involves cytoplasmic continuity between cells, and relies on plasmodesmata (PD), small cytoplasmic pores located in the cell walls. Few genes controlling PD formation have been characterized, despite the crucial role that PD play in regulating symplastic movement. Apoplastic transport entails uptake from the extracellular space, or apoplast. In maize, sucrose is the primary transport sugar. It is symplastically translocated from the PS cells into the vascular parenchyma (VP) cells in the vein. Subsequently, VP cells export sucrose into the apoplast, and in turn the sieve element-companion cell complexes import it.

The tie-dyed2 (tdy2) mutation conditions a yellow and green variegation pattern in leaves. Excessive carbohydrate accumulation distinguishes yellow regions from normal-appearing green regions and nonmutant leaves. Radiolabeling experiments with C14-sucrose suggest this accumulation occurs because of a transport defect between PS cells and the phloem. Thus, the tdy2 defect could affect either symplastic or apoplastic sucrose movement in the leaf. Previous studies using transmission electron microscropy (TEM) indicated no defects were present in the PD between the PS and VP cells. To test whether the tdy2 mutation has an impact on PD function after the apoplastic phloem loading step, we are examining PD frequency and structure between the sieve elements and companion cells. Preliminary results suggest that PD frequency is unaffected in veins of tdy2 yellow leaf regions relative to nonmutant tissues, but that PD structure between the sieve elements and companion cells is defective. These results potentially implicate Tdy2 function in the genetic control of PD formation, providing insight into a fundamental process critical to plant growth.

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P24

Poster Removed

P25

Influence of Cryptic Population Structure on Observed Mating Patterns in the Wild Progenitor of Maize (*Zea mays* ssp. *parviglumis*)

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Although various studies have measured pollen flow of cultivated maize in agricultural settings, little is known about the dynamics of pollen flow in natural populations of teosinte. Basic information regarding teosinte mating system parameters and how they are impacted by landscape and land use will inform both population and conservation genetics of the taxa. Indirect two-generation analysis of pollen flow has been an effective alternative to exhaustive paternity analysis for providing such data in large, high-density plant populations. In this study, over a thousand plants from a single large population of the teosinte *Zea mays* ssp. *parviglumis* were genotyped at ten microsatellite loci to assess genetic diversity, mating system parameters, and patterns of pollen flow. Subpopulations inferred by spatially-explicit Bayesian assignment had markedly different parameter values than those assessed for the entire population. Failure to account for subpopulation structure would have resulted in an overall underestimate of pollen dispersal distance. Pollen dispersal distribution curves were consistently thin-tailed, indicating a low level of long-distance dispersal. This may explain previously identified fine-scale genetic structure in *Zea mays* ssp. *parviglumis* populations and suggests the species could be particularly susceptible to barriers of dispersal across a landscape. Our results underscore the importance of considering cryptic structure while estimating pollen flow and recent demography. Importantly, conservation effort for teosinte should seek to preserve population connectivity to maintain the genetic integrity of this potentially important crop-breeding resource.

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P26

An integrated expression profiling system for maize

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The sequenced maize genome offers the opportunity to conduct highly parallel expression profiling using "all genes" platforms. We describe an integrated expression profiling system for maize based upon creation of a new Affymetrix GeneChip built from evidence-based gene predictions of the B73 nuclear, chloroplast and mitochondrial genomes. The expression of each gene is assayed by 25 x 25nt oligo probes distributed optimally across the exon space, allowing quantification of transcript levels, interrogation of splicing, and maximized coverage for discovery of sequence polymorphisms. To add value to the generated data, statistical and visualization tools will supported by the PLEXdb. PLEXdb features MIAME-compliant experiment annotations as well as required Plant Ontology terms through PLEX Express, its user-friendly, web-based submission tool. For every probe set on the chip, links to genome resources in PlantGDB, Uniprot and NCBI annotations, as well as genome alignments MaizeGDB, Maizesequence.org and Gramene.org will promote the integration of genetic, physical, and expression resources for grass genomics.

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P27

Analysis of the Maize Gametophytic Transcriptomes

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Both male and female gametophytes play crucial roles in sexual plant reproduction, generating cells (egg, central cell and sperm cells) to carry out fertilization. Gene expression during the haploid, gametophytic stage, presumably produces the proteins necessary to carry out the characteristic patterns of growth and development that precede fertilization. As a complement to a large-scale screen for gametophytic mutants that should help identify gametophytically-required genes, we are producing RNA-seq data using the Illumina platform, to characterize gametophytic transcriptomes, at both the qualitative and quantitative levels. Although the male gametophyte transcriptome is fairly well characterized in a few plants, the relative inaccessibility of the female gametophyte has limited the availability of genome-scale data on this structure. We have taken advantage of the relatively large size of the maize female gametophyte to generate replicated RNA-seq libraries from dissected embryo sacs, comparator ovules, mature pollen, and seedlings (as a baseline) of the B73 inbred. We aligned paired-end and single RNA-seq reads to the maize genome sequence (4a.53) using bowtie and blat and generated empirical transcripts and RPMK expression data using tophat and cufflinks. We also compared our empirical transcripts to the predicted transcripts to explore patterns of novel differential splicing.

Funding acknowledgement: National Science Foundation (NSF)

P28

COGENFITO: a new module of MaizeGDB to optimize isoline selection in breeding schemes

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Determining the phenotypic consequence of natural allelic variation at particular loci is a common goal of plant breeders and geneticists. Careful use of genetic marker data can reduce the number of generations required to create stock populations to address this need. When several-to-many QTL affect a trait, assessing the additive, dominance, and interaction effects of a single QTL requires a breeding approach. Traditionally, contrasts of nearly isogenic line (NIL) pairs were used to eliminate variation at non-target loci. In previous work, we demonstrated that effectively isogenic line (EIL) pairs can be useful as well. EIL pairs fix the alleles at “control loci”, allowing the alleles at “target loci” to vary. Because the use of EILs alleviates the need for introgressions, substantial breeding time is spared.

We have released COGENFITO, a composite genotype finder tool at MaizeGDB that serves as an interactive browser for public genotype dataset. COGENFITO helps researchers efficiently sort and sift through marker data to identify lines with user-defined haplotypes. Currently, COGENFITO interrogates genotype data for 26 RIL populations (IBMRIL + NAM) whose seed are available from the MGCSC. Researchers specify the mapping population and desired genotypes at markers of interest. Browsing the genotype data associated with particular local areas of maps is facillitated by a “show centiMorgan range” function. After a query is submitted, an output screen shows which stocks match the desired parameters. A color-coded graphical display of haplotypes facilitates visual and intuitive selection of isolines for experimental breeding. Germplasm-specific hyperlinks to the MGCSC expedite the process of obtaining desired lines for inclusion in experiments. COGENFITO is applicable to a wide range of research pursuits, including QTL cloning, dissection of epistatic interactions, and control of genetic background in selection studies.

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P29

Diversity of maize and its wild relatives in its world centre of origin and diversity

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During the last three years the National Commission for Knowledge and Use of Biodiversity (CONABIO) has coordinated collecting of accessions and compiling information from maize and its wild relatives in Mexico through 13 projects carried out by several national institutions along the country. Product of this effort was to develop a database (using Biotica 5.0) that contains 21290, 610 and 537 records of maize, teocintle and *Tripsacum* spp., respectively. The most relevant issue of this national effort are: documenting a higher richness and variation inside and among races of maize, and the collecting of new populations that possible are new races; recording new localities for teocintle populations at the Balsas wathersed; discovering a new population of wild perennial teocintle in a dry forest, which maybe a new species for the science; describing a new species of teocintle for the wet tropics; and, the confirmation of the broad distribution of *Tripsacum* genera along the country.

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P30

Development and Validation of High-throughput Resistance Gene Analog (RGA) SNP assays in Maize

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Resistance gene analog (RGA)-based molecular markers have been widely utilized by researchers during last two decades in their efforts to map and clone disease resistance genes in plants. However, majority of those markers, including sequence tag sites or cleaved amplified polymorphic sequences, are low-throughput and are not suitable for large scale application in industrial units. Single nucleotide polymorphism (SNP) markers have proven to be high-throughput and amenable to automation. One of the most popular genotyping chemistries, Illumina GoldenGate (GG) assay, is capable of multiplexing up to1536 SNPs and validating them within three days. However, GG assay is very sensitive to duplicated sequences. RGAs are members of multigene families, have resulted from multiple duplicated events and represent paralogues. In this study, we used public maize RGA sequences and applied inexpensive in silico method to detect electronic SNPs (eSNPs). Virtual SNPs were validated using GG assay. The results of our experiments demonstrated the feasibility of development GG SNP assays from RGA sequences representing different classes of R genes. GG-validated SNPs will serve as valuable markers for high-throughput marker-assisted selection of maize R genes and their eventual cloning.

P31

Following tetraploidy in maize, a short deletion mechanism removed genes preferentially from one of the two homeologs

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Previous work in Arabidopsis found that, following an ancient tetraploidy, genes were lost preferentially from one of the two resulting homeologs. The loss of duplicate genes copies following a whole genome duplication is known as fractionation. The mechanism of fractionation was unknown.

Sorghum and maize, both sequenced, share a grass ancestor about 12 MYA. Shortly thereafter, the maize lineage survived a tetraploidy, and its fractionation is recent enough that that many duplicate genes that are in the process of being lost are still present as fragments. We heavily masked every sequence in sorghum except exons and those other sequences shared orthologously with rice, and then asked what became of the duplicates of these sorghum-maize genes after tetraploidy?

We show that genes are lost, not relocated, and that single-gene loss by deletion is the rule. Maize is such a recent tetraploid that fractionating gene loss is still underway. By comparing maize exons that contain 7-178 bp single-event deletions with rice and sorghum orthologs, we infer that the sequences present before the deletion events were flanked by short direct repeats, a signature of intrachromosomal recombination. This deletion mechanism operated 2.8 times more frequently on one of the two maize homeologs, consistent with previous observations of biased fractionation. The over-fractionated homeolog is also targeted for higher rates of transposon removal, but does not have a higher synonymous base substitution rate, nor could we find differentially placed methylation domains. We hypothesize that deletions, targeted by some as yet unidentified epigenetic signal, to one of the two homeologs, are the primary mutations on which purifying selection has acted.

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P32

Plant Genomic Resources at National Center for Biotechnology Information

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Plant genomics is a simple expansion of the scope of genomics at the National Center for Biotechnology Information (NCBI). In addition to the tools for storage of and analysis of INSDC nucleotide sequence such as, respectively, GenBank and BLAST, genomics at NCBI includes databases that enable 1) monitoring the progress of genome sequencing projects (Entrez Genome Projects), 2) datamining of probes (Entrez Probes, UniSTS), 3) datamining of gene information (Entrez Gene), 4) defining gene-specific sets of cDNA by clustering (UniGene), 5) viewing genome units and the underlying components (MapViewer, CloneDB/CloneFinder) and 6) databases that allow datamining of NCBI-generated data (RefSeq and Plant Protein Clusters). These standalone tools are enhanced at NCBI by the capability to move among these and other databases as the data associations dictate. The pan-organism resources are supplemented by plant-specific resources: plant text search, PlantBLAST, and plant-EST BLAST. PlantBLAST provides organism-specific databases composed solely of the accessions associated with mapped loci visible through MapViewer. EST-BLAST provides plant-specific databases composed solely of the ESTs from those plants with more than 40,000 ESTs.

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P33

MaizeGDB: Tools And Resources

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MaizeGDB (http://www.maizegdb.org) is the research community's database for maize genetics and genomics. This demonstration presents the latest updates to MaizeGDB with the focus on integration with sequence, especially the maize pseudomolecules. These updates include three new tools: the Locus Lookup, the Locus Pair Lookup, and MaizeGDB's new BLAST interface; as well as a new project called POPcorn. The Locus Lookup tool allows researchers to identify a genomic window within the MaizeGDB Genome Browser that should contain a given locus for which direct alignment, probe data, or genetic map information is known. The Locus Pair Lookup allows researchers to find a genomic region based on specifying two loci on the same chromosome. MaizeGDB's new BLAST interface is integrated into the MaizeGDB Genome Browser and existing record pages. Researchers can BLAST a sequence and visualize the results on the pseudomolecules with the MaizeGDB Genome Browser. POPcorn is a new project ancillary to MaizeGDB that serves as a portal to ongoing maize project resources as well as a centralized location for BLASTing cooperating resources' sequence-indexed data. These new additions are designed to make MaizeGDB a more sequence-centric resource.

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P34

MaizeGDB -- The Data

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We provide an overview of data currently in MaizeGDB, the recent additions and how the data are integrated for linking to the genome browser. Notable new data in 2009 included (1) *Mu* insertion points, flanking sequences and stocks (Uniform MU project, University of Florida, Gainseville, FL); (2) MAGI based SNPs, genetically mapped and aligned to the B73 sequence [Liu, S et al 2009 *PLoS Genet.* 5:e1000733; Liu, S et al. 2010 *Genetics* 184:19-26]; and (3) alignment of genetically mapped and sequenced loci from IBM2 2008 Neighbors to B73 RefGen_v1 [Wei, F et al. *PLoS Genet.* 2009 5:e1000715]. Other alignments contributed directly for the MaizeGDB Genome Browser will be described on a separate poster. Consistent with advice from our Working Group meeting 2009, we renewed emphasis on the curation of empirically confirmed maize gene function and phenotypes, and, importantly, the engagement of maize cooperators in this effort. Some 100 new functionally defined genes have been added to MaizeGDB, along with updates of 300 genes that were previously listed. Linking the functional information to the Genome Browser is easily accomplished for sequenced genes. In some cases, genetic map locations are useful, in particular for regions not yet sequenced or, if sequenced, are poorly oriented or unanchored to chromosomes. MaizeGDB continues to update IBM2 2008 Neighbors maps with these loci, to support tools such as Locus Lookup (Andorf et al. 2010 *Bioinformatics* 26:434-436). We invite and respond positively to volunteered participation and suggestions from the community about data content. Please use links on each page of MaizeGDB, or contact the ***maize gene review*** (www.maizegenereview.org), a new online, peer-reviewed journal with the express purpose to engage the community in curation of gene function. The journal is sponsored by the Maize Genetics Cooperation Newsletter and was first introduced at the 2009 Maize Meetings. Persons with larger projects and who wish MaizeGDB to accept their data are advised to contact Carolyn Lawrence well in advance of submitting grant proposals.

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P35

How to use the new sequence-based functionalities MaizeGDB

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To make maize sequence more accessible and useful within its genomic context, the MaizeGDB Team has incorporated a GBrowse-based Genome browser and a new "Locus Lookup Tool" that add sequence-oriented utility to the vast genetic datasets available at MaizeGDB. Because it is difficult for individual researchers to keep abreast of all the sequence updates, we have made outreach and education a priority. In this poster we provide case studies on how to use MaizeGDB's new features. In addition, short movie tutorials, PowerPoint tutorials and problem sets and answers suitable for individual researchers and classroom use are available at http://outreach.maizegdb.org, accessible from the home page at http://www.maizegdb.org under tutorials (check out new content on B73 refgen_vX!) Input from maize researchers on transitioning all useful sequence-based information into MaizeGDB is needed, so please stop by and let us know what you want to see!

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P36

POPcorn: A PrOject Portal for corn

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The impressive growth of maize data and information available on the Internet means that locating and searching for relevant data and information is increasingly difficult for maize researchers. Finding appropriate sites then learning how to navigate the sites can be difficult and time-consuming. Finding information associated with a particular sequence is likewise challenging, sometimes involving BLASTs at multiple sites and database searches that require in-depth knowledge of the database architecture and other technical information. In addition, data generated by projects can be lost after the completion of the project, or left untouched, only to degrade over time.

The POPcorn project is addressing these problems by providing a set of search utilities for locating maize projects and online resources, BLASTing against multiple sequence targets from multiple sources, and for searching sequence-indexed data: data linked to nucleotide and/or protein sequences. In its next stages of development, POPcorn will also provide pipelines and processes for migrating project data to MaizeGDB for long-term storage.

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

P37

Population structure of *Zea mays* ssp. *parviglumis*

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Knowledge of population structure and demographic history are essential to understanding genome-wide patterns of molecular variation. Here we present initial results from a range-wide study of diversity in teosinte, *Zea mays* ssp. *parviglumis*. We sampled ~1000 individuals of *parviglumis* from across its range, including relatively deep sampling (>20 individuals) of 25 populations. Individuals were genotyped for 983 SNPs using the Sequenom MassARRAY System. We find surprisingly similar overall levels of diversity among populations in the Balsas region, but see some evidence for differences in the frequency spectrum of polymorphisms among populations, possibly reflecting past demographic change. Our data also reveal stronger population structure than previously reported for parviglumis. We discuss the diversity and structure data in terms of the demographic history of *parviglumis* and its implications for conservation of the taxon.

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P38

SAMgm: An Information Resource for the Functional Annotation of Maize Genes

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An ongoing series of investigations has identified a set of over 7,000 *Zea mays* genes of interest in shoot apical meristem (SAM) function and leaf primordial development. We have developed and deployed a web-based application (http://sam.truman.edu) to facilitate the functional annotation of these genes, including the automated harvesting of gene ontology (GO) numbers and enzyme information for gene products with known EC numbers (http://www.genome.jp/kegg/). The application automates several searches that were previously performed manually by researchers including accessing multiple BLAST reports (both from NCBI and InterPro), harvesting the longest maize EST contigs (http://magi.plantgenomics.iastate.edu/) and locating maize gene models (http://www.maizesequence.org). Abstracts from PubMed, with evidence codes, can also be entered into individual gene pages following literature searches. Work is underway to merge and link the information with the GeneWiki system managed by Gramene (http://plantgenewiki.gramene.org/genewiki/index.php/Main_Page).

This is a second-generation system developed using Ruby on Rails and supported by a MySQL database backend. The database schema is a modified version of the Chado schema (http://gmod.org/wiki/Chado) and thus is designed to be highly interoperable with other genomic databases. The display of information is standardized and customizable for ease of use.

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P39

The pan-grass synteny project

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Currently, four grass species are represented by at least one complete genome sequence: brachypodium, maize, rice, and sorghum. These genomes provide a powerful comparative dataset for understanding the evolution of phenotypic differences and differences in chromosomal structure. However, identifying syntenic regions among these genomes is complicated by a whole genome duplication (WGD) event that happened prior to the radiation of the grasses, and a maize specific WGD that happened soon after the divergence of the maize and sorghum lineages.. Potentially, this creates syntenic gene-sets that include ten genes: two genes from brachypodium, rice, and sorghum, and four from maize. However, both fractionation and subfunctionalization occur following a WGD, so many complexities arise. In order to correctly classify syntenic relationships within and among these genomes, we developed an automated system that, for any two genomes, infers syntenic regions by identifying collinear sets of putatively homologous genes, calculates the synonymous mutation (Ks) rates for all protein coding syntenic gene-pairs, merges together small blocks of synteny, and calculates the mean Ks value of these merged blocks. This method permits the unambiguous assignment of every syntenic region between all pair-wise comparisons of these genomes, except for one region in all grass genomes that appears to be undergoing extensive concerted evolution. These pair-wise comparisons and evolutionary event assignments are condensed into datasets that enable researchers to easily find the syntenic orthologs and homeologs of their gene of interest. To make these data readily assessable, links are built into the CoGe suite of web-based comparative genomic tools for rapidly extracting sequence data, and comparing multiple syntenic regions to identify different types of genome evolution including local gene duplication, fractionation, conserved noncoding sequences, transpositions, and inversions, as well as gene annotation errors.

CoGe is publicly available at: http://synteny.cnr.berkeley.edu/CoGe

Introduction for maize whole genome analysis using CoGe:
http://synteny.cnr.berkeley.edu/wiki/index.php/Maize_Sorghum_Syntenic_dotplot

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P40

Two Types of Meiotic Crossovers Coexist in Maize

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Meiotic crossovers (COs) have been demonstrated to be formed through two distinct pathways in Arabidopsis and tomato. Pathway 1 is ZMM-dependent and produces interfering COs, so their distribution along the chromosome is more regular than random. Pathway 2 is Mus81-dependent and presumably produces randomly distributed COs. Through a modeling approach, we investigated these pathways in maize (Zea mays L.) via the distribution of late recombination nodules, which indicate CO positions along the synaptonemal complex. Interference was modeled using both the “statistical” gamma model and the “mechanical” beam-film model. For each chromosome, we exclude at a 98% significance level the hypothesis that a single pathway underlies the formation of all COs, pointing to the co-existence of two types of crossing-over in maize. We estimate the proportion of COs coming from the non-interfering pathway to range from 6% to 23% depending on the chromosome, with a cell average close to 15%. The mean number of non-interfering COs per chromosome is significantly correlated with the length of the synaptonemal complex. We also quantify the intensity of interference in the first pathway and find it lies within ranges previously estimated in other organisms. Finally, we develop inference tools that allow one to tackle, without much loss of power, complex CO interference models such as the “beam film”. Until now, estimation of parameters was not possible with such models because no likelihood function can be derived. This advance will allow more realistic mechanisms of CO formation to be modeled in the future.

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P41

Using NAM polymorphism data to identify molecular markers in Corn Belt Dent inbred lines.

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Molecular polymorphisms are critical to map Mendelian and quantitative traits. Recently, set of nearly 500,000 single nucleotide polymorphisms (SNPs) were identified within the 27 diverse inbred founders of the maize nested association mapping (NAM) population[1]. We tested the utility of these SNPs as genetic markers for a diverse set of maize Corn Belt Dent inbred lines using the iPLEX Gold SNP genotyping assay on the Sequenom MassARRAY Platform. We describe a novel design pipeline which integrates the NAM HapMap data with the most recent B73 genomic sequence, allowing us to design a SNP-based assay from gene common names or microarray oligonucleotide sequences. An assay of 3 NAM and 11 non-NAM inbred lines revealed that any two inbred lines were on average polymorphic at 37% of the candidate SNPs, higher than the expected frequency of SNPs based on the NAM founders. Polymorphisms were relatively uncommon among very closely related lines (e.g. OH43 and A619), as expected. We also correlated attributes of NAM SNPs (exonic versus intronic, functional class of gene) with the frequency of polymorphism detection. These results demonstrate the applicability of the NAM HapMap data to non-NAM genotypes and indicate the utility of this data for establishing genotyping assays for diverse maize genotypes.

1. Gore, M.A., et al., *A first-generation haplotype map of maize.* Science, 2009. **326**(5956): p. 1115-7.

P42

A functional maize centromere without CentC and CRM sequences

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The centromere is the part of the chromosome that organizes the kinetochore, which mediates chromosome movement during mitosis and meiosis. In most multicellular organisms, the centromere region is composed of species-specific sequence repeats arranged in large tandem blocks. Maize centromeres contain two basic types of DNA elements. One is the centromeric satellite referred to CentC, a 156 base pair unit that is present at all of the primary constrictions of the chromosomes. Another one is a retrotransposon family referred to as Centromeric Retrotransposon of Maize (CRM). A small fragment from chromosome 3 was created by irradiation by Stadler and Roman and named Duplication 3a. It is interesting that this small chromosome does not contain any detectable CentC and CRM sequences. When molecular features of functional centromeres such as CENH3 and CENP-C were examined, this small chromosome contained them, even without CentC and CRM sequences. Immunolocalization analysis of phosphorylation of Ser-10 of histone H3 on this small chromosome indicated that there were no detectable differences in the level of phosphorylation of Ser-10 of histone H3 between the maize normal chromosomes and this small chromosome. Meiotic analysis revealed that sister chromatids divided equationally at meiosis I as do all small chromosomes examined to date in maize. These observations add further evidence for the epigenetic nature of centromere specification in maize.

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P43

Coix centromeres contain evolutionary conserved repetitive DNA sequences

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The genus Coix, belonging to Maydeae, is the closest group to the genus Zea after Tripsacum. It consists of 4-7 species with different levels of ploidy, such as diploids (2n=10), tetraploids (2n=20), hexaploids (2n=30) and octoploids (2n=40) on the basic chromosome number x=5. Here, we report the sequences of one centromere-associated bacterial artificial chromosome clone from a Coix lacryma-jobi (2n=20) library. Two Ty3/gypsy-class retrotransposons, CRC (centromeric retrotransposon of C. lacryma-jobi) and PCRC (peri-centromeric retrotransposon of C. lacryma-jobi), and a (peri)centromere-specific tandem repeat with a unit length of 153 bp were identified. The CRC is highly homologous to centromere-specific retrotransposons (CR) reported in grass species. An 80-bp DNA region in the 153 bp satellite repeat was found to be conserved to centromeric satellite repeats from maize, rice and pearl millet, which diverged from C. lacryma-jobi millions of years ago. Fluorescence in situ hybridization (FISH) showed that the three repetitive sequences were located in (peri-)centromeric regions of both C. lacryma-jobi and C. aquatica. However, the 153 bp satellite repeat was only detected on 20 of the 30 chromosomes in C. aquatica. Immunostaining with an antibody against rice CENH3 indicates that the 153 bp satellite repeat and CRC are both the major components for functional centromeres, but not all the 153 bp satellite repeats or CRC sequences are associated with CENH3.

P44

Dynamics of Mitochondrial DNA Insertions into Maize Chromosomes

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The transfer of mitochondrial DNA (mtDNA) into nuclear genomes occurs continually in flowering plants. We have examined the mtDNA insertion sites of maize using fluorescence *in situ* hybridization (FISH) of mtDNA onto metaphase chromosomes from root tips. The size and distribution of mtDNA insertions varies among maize inbred lines, further increasing the chromosomal diversity within maize. In B73, one insertion on 9L contains a majority of the maize mitochondrial genome. A similar site was found on 9L in M825, a sweet corn line, and HP301, a popcorn line. A much smaller insertion, containing a portion of the mitochondrial genome exists in many other inbreds at this location. Because the three lines with large insertions on 9L are unrelated, we believe that additions of mtDNA occurred independently through similar mechanisms at this site. The available sequence for B73 was analyzed for the 9L large mtDNA insertion. Fiber-FISH has been used to compare the sequence data to the mtDNA site present in the B73 nuclear genome. Preliminary results suggest that the B73 site is approximately 2 Mb in size, indicating that multiple copies of portions of the mitochondrial genome are present.

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P45

In vivo visualization of chromosome movement using protein tethering

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Protein tethering has been used in many different applications to study chromatin proteins. Four different DNA binding modules, LacI, LexA, Gal4 and TetR are known to be functional in Arabidopsis for this purpose. We are testing the utility of each of these modules, as well as a fifth derived from human centromere binding protein CENPB in maize. To this end we have developed a specialized in vivo system to determine their binding affinity. A 156bp monomer containing one copy of each binding motif was amplified into very long tandem repeat arrays using overlap extension PCR, and biolistically transformed into maize. The arrays inserted as intact single loci on chromosomes 3, 4 and 7 (abs3, abs4 and abs7). The ~200kb abs arrays contain as many as 1000 monomers each and are easily visualize by FISH. Transient assays in cultured cells will be used to compare the binding of each module using fused fluorescent tags. We are also using the tethering system to visualize and modify chromosome movement. In one application we have fused the Gal4 binding domain to the kinesin protein NCD (a microtubule-based motor protein) to determine how induced chromosome arm motility affects meiosis and chromosome transmission.

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P46

Mechanisms underlying reduced transmission of B-A chromosome segments from tertiary trisomic stocks

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Using B-A translocations, a set of tertiary trisomic lines has been created such that each line contains one extra chromosome (a “trisome”) that encompasses a large portion of a chromosome arm (Auger and Birchler 2002 J Hered. 93: 42-47). Currently, 16 of the 20 chromosome arms of maize are available in this set. These aneuploid stocks are relatively stable, and the presence of a kernel marker on each trisome facilitates analysis of their heritability. In fact, Auger and Birchler (2002) noted that almost all trisomes in their original set showed reduced transmission through the male, and many showed reduced transmission through the female. One hypothesis to explain the reduced transmission of the trisome is impairment of gametophyte function by increased dosage of the chromosome arm involved, leading, for example, to a reduced ability of aneuploid pollen to compete with wild-type pollen. Alternatively, the trisomic chromosomes could have a reduced ability to be segregated into the appropriate meiotic products. We have investigated the effect of this set of trisomes on male and female gametophytes, to determine whether any observed phenotypic differences could explain the reduced transmission. For the male gametophyte, we found that the presence of the duplicated chromosome arm in the pollen grain impaired function and/or development of the pollen for 11/16 of the trisomic lines. These data are consistent with a deleterious effect for these particular trisomes, expressed during pollen development, which subsequently leads to reduced transmission of the trisome. In the female gametophyte, even trisomes that have the lowest female transmission have little to no corresponding reduction in fertility (i. e. embryo sacs are fertilized efficiently), indicating that the presence of the trisome is not detectably deleterious to embryo sac function. This suggests that the mechanism(s) responsible for reduced trisome transmission through the female are distinct from those in the male.

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P47

Meiotic mutants affecting homologous synapsis

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Meiosis occurs in cells that have been developmentally targeted to become meiocytes. This process relies on at least one gene, mac1 (Sheridan, et al.1996). This cell fate determination step is followed by an alteration of the somatic cell division cycle into a meiotic cell cycle. The Ameiotic1 gene is required for this change. To ensure separation of homologs at the first reductional division, homologous chromosomes pair and synapse and undergo homologous recombination to form chiasmata. The Afd1 gene affects all these events, and is essential for reductional segregation of chromosomes.

The majority of the mutants we have identified affect both homologous pairing and synapsis. Since we have antibodies against many proteins of the Synaptonemal Complex (SC), such as ASY1/HOP1, AFD1/REC8 and ZYP1, and the RAD51 protein essential for recombination, we have studied their presence and behavior in our mutants. The Dsy2 gene, a genuine synaptic mutant, is required for loading ZYP1 protein onto chromosomes and formation of the central element of the SC. The mtm99-14 mutant is defective in lateral element formation although two lateral element components, ASY1/HOP1 and AFD1, are present. A unique ms*N2415 mutant manifesting abnormal synapsis and segregation is defective in chiasmata resolution.

In addition a novel mutant with a divergent spindle phenotype similar to the well-known dv1 maize mutant (Clark, 1940) has been identified recently from an EMS screen.

Funding acknowledgement: National Institutes of Health (NIH)

P48

Now is this the *Ph1* gene?

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(*Ph1*) (*Pairing homoeologous 1*) locus has been at the heart of wheat research since its discovery in early 1950s but the identify of the underlying gene(s) has been illusive. High-density physical mapping of the previously identified *Ph1* gene region unambiguously assigned the *Ph1* locus to a 2.5Mb wheat BAC scaffold, carrying 93 genes, and bracketed by the deletion breakpoints of 5BL-1 and ph1c. Localization of other well known meiotic genes to the same 5L0.5 region suggested that the region carries a battery of meiotic genes, including *Ph1*, *DMC1* and *ASY1*, and other uncharacterized genes. To characterize these meiotic genes including *Ph1*, we first short-listed the 93 candidate genes first based on their function and/or expression and then based on domain/motif comparisons. The 12 selected genes (including *TaDMC1*, *TaASY1* and *TaCDC2-4*- previously claimed Ph1 gene candidate) were evaluated by virus-induced gene silencing (VIGS). Results of gene silencing of *TaDMC1* showed an average of 37.17 univalents compared to 21 bivalents for controls, whereas VIGS silencing of *TaASY1* showed multivalents. VIGS analysis of one of the *Ph1* region genes (*TaWSU-1*) showed formation of quadrivalents/higher order pairing upon silencing, similar to that observed for the ph1 mutants and the 5BL deletion lines missing the *Ph1* gene. Except for another gene (*TaH51L*) that showed an average of 4 univalents and 19 bivalents, all the other *Ph1* region genes only showed 21 bivalents. Gene expression analysis by qRT-PCR of the plants showing increased pairing by VIGS of *TaWSU-1* showed reduction in expression by 30 to 50%. These findings suggested that the candidate gene *TaWSU-1* represents a novel meiotic gene that influences diploid like pairing behaviour of the hexaploid wheat, very similar to that of the *Ph1* locus.

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P49

Characterizing development...

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Development in a maize seedling has been historically described by leaf or leaf-tip stage. The underlying assumption is that plants at the same leaf-tip stage are also at the same developmental stage with regard to other aspects of development. We have found that this underlying assumption is false. Maize seedlings at the same leaf-tip stage exhibit considerable plant-to-plant variability: whole plant dry matter varies by 2- to 3-fold; and perhaps more importantly ear initial size varies by 2-fold. Preliminary observations suggest that developing ear initials are variable in more than just size, that these differences in size reflect differences in developmental stage. There are 4 types of reproductive meristems in ear development: inflorescence meristem (IM), spikelet pair meristem (SPM), spikelet meristem (SM) and floret meristem (FM). Shorter ear initials tend to be at the earlier stages of development (i.e.,SPM and SM stages), while longer ear initials appear to be at the later stages of development (i.e., SM or FM stages). To further understand the extent and magnitude of plant-to-plant variability on ear initial development, we are following the expression of 4 genes whose expression is present at specific stages of ear initial development: *ramosa1 (ra1), ramosa2 (ra2), branched silkless1 (bd1)* and *zea agamous1 (zag1)*. The preliminary results regarding the characteristics of plant-to-plant variability and ear development will be presented.

Funding acknowledgement: Ontario Research Fund (ORF), Syngenta Company, Natural Sciences and Engineering Research Council of Canada (NSERC)

P50

emb8522 encodes a chloroplast-targeted pentatricopeptide repeat (PPR) protein necessary for maize embryogenesis

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The large family of pentatricopeptide repeat (PPR) proteins is characterized by degenerate 35 amino acid repeat motifs that constitute RNA binding domains. In plants the majority of the family members are predicted to be targeted to mitochondria or chloroplasts, where they are involved in post-transcriptional processing of organellar RNAs. Here, we demonstrate that a mutation in *PPR8522* confers an *emb (embryo-specific)* phenotype: while mutant endosperms have normal morphology, mutant embryos are severely affected. As early as 3 days after pollination they show a reduction in cell number in comparison to wildtype siblings and present morphological aberrations that are quite variable between individuals and genetic backgrounds. Mutant plantlets generated by embryo rescue experiments have an albino phenotype and cease growth around the 4 leaf stage, indicating that the mutation *emb8522* also affects chloroplast functioning and general plant growth. The *emb8522* mutation was isolated in an active *Mutator (Mu)* population, and perfect co-segregation in a segregating population of 431 individuals suggested that it was caused by a *MuDR* insertion in the first exon of a PPR gene (PPR8522). Supportive evidence of molecular cloning was provided by fine mapping that confined the *emb8522* mutation to a 110 kb region in the last BAC of chromosome arm 1L, in which there are no genes models except PPR8522 (GRMZM2G042883). The deduced PPR protein is 875 amino acids in length, contains 13 PPR repeats, is predicted to be targeted to the chloroplast and represents the first PPR protein rigorously associated with an early-lethal phenotype in maize.

P51

sterile tassel silky ear1 (*sts1*) Regulates Stamen and Lodicule Identity

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Angiosperm flowers consist of four concentric whorls of organs: sepals, petals, stamens, and carpels. The identity and patterning of these whorls has been described in the classic ABC model of flower development and is primarily controlled by the combinatorial action of MADS-box transcription factors. The grasses have flowers with highly derived outer (sterile) organs compared to the model eudicot species used to generate the ABC model, and as monocots are evolutionarily distant from the eudicots. Consequently, an investigation of floral organ identity and patterning in grasses should provide insight into how much of the ABC model is conserved across the angiosperms. In a forward genetic screen for maize floral mutants we isolated a novel mutant in which the second (lodicule) and third (stamen) whorls of tassel florets are consistently transformed into bract-like organs, which we have called *sterile tassel silky ear1* (*sts1*). Interestingly, the ear florets of sts1 are distinct from the tassel and have a conversion of stamens to carpels rather than bracts. Mapping and sequence data indicate that sts1 is likely a loss of function in the maize *PISTILLATA* (*PI*) ortholog *Zmm16*. We have generated *Zmm16*pro>>*Zmm16-YFP* transgenic plants that recapitulate the known expression of *Zmm16* but provide even better resolution revealing that *Zmm16* expression is lacking from the region of the floral meristem where the medial lodicule is missing in many (but not all) grass species. This is the first described *PI*-like mutant in the grasses and will help to further characterize the genetic control of their unusual morphology of grass florets.

Funding acknowledgement: National Science Foundation (NSF)

P52

A cellular study of teosinte *Zea mays* ssp. *parviglumis* (Poaceae) caryopsis development showing several processes conserved in maize

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The evolutionary history of maize (*Zea mays* ssp. *mays*) is of general interest because of its economic and scientific importance. Here we show that many cellular traits described previously in developing caryopses of maize are also seen in its wild progenitor teosinte (*Zea mays* ssp. *parviglumis*). These features, each with a possible role in development, are: (1) an early programmed cell death in the maternal placento-chalazal (P-C) layer that may lead to increased hydrolytic conductance to the developing seed; (2) accumulation of phenolics and flavonoids in the P-C layer that may be related to the antimicrobial activity; (3) wall-in-growth formation in the basal endosperm transfer layer (BETL); (4) localization of cell wall invertase in the BETL, which is attributed to the increased transport capacity of photosynthates to the sink; and (5) the endoreduplication in endosperm nuclei suggested to contribute to increased gene expression and greater sink capacity of the developing seed. In maize caryopsis, these cellular traits have been previously attributed to domestication and selection for larger seed size and vigor. Given the conservation of the entire cellular program in developing teosinte caryopses described here, we suggest that these traits evolved independent of domestication, and predate human selection pressure.

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P53

A negative regulator of aleurone development functions downstream of *dek1*

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Cereal endosperm represents one of the most important plant tissues for human well-being. Understanding how various cell types are specified could allow the manipulation of seed properties. Our lab is investigating the genetic regulation of aleurone cell fate acquisition. Several known genes, including *cr4* and *dek1*, are positive regulators of aleurone fate because the recessive loss-of-function mutants lack aleurone cells but rather contain starchy endosperm cells in the peripheral layer. The *thick** (*thk**) mutant identifies a negative regulator of aleurone identity because the loss-of-function mutant has an abnormally thick aleurone layer 4-6 cells deep, compared to the single layer in wild type. The *thk** mutant is epistatic to *dek1*; double mutants show the thick aleurone phenotype. *thk** mutant sectors show sharp boundaries suggesting the gene function is cell autonomous. The mutant is associated with a deletion of approximately 2 megabases on chromosome 1. Experiments are ongoing to identify the specific gene within the deleted region responsible for the mutant phenotype.

A second mutant of interest is *naked endosperm** (*nkd**) which shows defects in aleurone cell differentiation and in the specification of aleurone cell layer number. Mutant cells lack the dense granular cytoplasm typical of normal aleurone cells and mutants also contain approximately 3 cell layers instead of one. Thus, dual functions are potentially affected. This mutant shows a 15:1 F2 segregation ratio suggesting it identifies duplicate gene functions. Genetic mapping has identified syntenic genes encoding putative zinc finger proteins as likely candidate genes. Current research is directed at testing this hypothesis.

Funding acknowledgement: United States Department of Agriculture (USDA)

P54

A role in leaf epidermal development for Cellulose Synthase-Like D1

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Cellulose Synthase-Like D (*CslD*) genes remain among the few classes in the Cellulose Synthase Superfamily whose function has not been demonstrated. They contain domains characteristic of enzymes that synthesize β-linked cell-wall polysaccharides, such as cellulose, hemicellulose, or other cell wall components. We have identified transposon-induced mutations in *ZmCslD1* from the UniformMu (University of Florida) and TUSC (Pioneer Hi-Bred Int.) maize populations. Plants homozygous for Mu inserts in *ZmCslD1* develop a phenotype in which individual epidermal cells and groups of cells balloon out of the leaf surface during and after organ maturation. A rough texture results, especially on the abaxial surface. Individual cells expand up to 100 times their normal size. Cellular ballooning progresses from single cells to neighboring cells, resulting in large areas that take on a distinctive bumpy appearance in mature leaves. Epidermal cells in mutant leaves show diverse defects in cell division. In addition, despite the narrower leaves of mutant plants (mean width 45% reduced), average epidermal cells of these leaf blades are 15% wider than wildtype cells. Overall growth of mutant plants is also reduced, with mature plant height decreased by 7% and total dry weight reduced by 45% on average. Cell wall composition was similar for mutant and wildtype samples of both whole-leaf fractions and epidermis only. Transcript profiling using qRT-PCR showed greatest levels of *ZmCSLD1* mRNA in very young leaves, with the maximal abundance prior to leaf emergence from the whorl.

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P55

Anthers in 3-D: cell division, morphology, and fate from primordium to microsporogenesis

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Despite extensive research on floral development, surprisingly little is known about the mitotic proliferation stage, when the germ cell lineage is specified from somatic stem cells within anther locules. Two competing models have been proposed to explain cell fate specification: cell lineage and final position. A comprehensive data set combining morphometry with cell division parameters allows us to address these models with new insights. W23 inbred anthers sampled over 21 days were stained with propidium iodide (PI), which labels nuclei, and whole anthers were imaged with confocal microscopy. Cell numbers, sizes, and shapes were measured; it is clear that the five cell types of the anther locule differentiate at different stages. Each cell type follows a unique program of cell expansion and division, yet strikingly, the mature anther never has gaps or bulges. Cell-type specific mitotic rates were calculated and confirmed using 5-ethynyl-2'-deoxyuridine (EdU), which labels S-phase nuclei. EdU positive cells were found in scattered patches along the length of the anther, unlike the zonation characteristic of roots and leaves. A comparison of morphometric parameters between B73 and W23 and between the upper and lower florets indicated near identity, suggesting that the hallmarks of anther cell fate determination are highly canalized. Furthermore, PI and EdU labeling of the *mac1* (*multiple archesporial cells1*) mutant, which has a defect in cell fate and has been used to support the positional model, documented a cell division phenotype in very immature anthers. We propose that these techniques represent a powerful tool for mutant screening and analysis. New theories about global signaling and symplasmic transport through plasmodesmata are considered in light of contemporary models of organ ontogeny in the grasses.

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P56

Analyses of WOX3 regulation and candidate target genes involved in mediolateral leaf development

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The *WUSCHEL1-related homeobox3 (wox3)* orthologs *narrow sheath1 (ns1)* of maize and *pressed flower1 (prs1)* of *Arabidopsis* perform redundant functions with the homologous *wox* genes *ns2* and *wox1*, respectively, during the development of mediolateral domains in leaf-like organs. Both *ns1* and *prs1* accumulate in two meristematic foci and function non-cell autonomously during the recruitment of lateral organ founder-cells.

A conserved non-coding sequence (CNS) is identified in *wox3* homologs from *Zea, Sorghum*, and *Arabidopsis* that is required for normal *prs1* expression and function in transgenic *Arabidopsis*. Sequences contained within the *prs1* coding region are also required for the unique pattern of *prs1* transcript accumulation within two meristematic foci. Previous laser microdissection-microarray analyses of the ns1 mutant meristem implicated auxin and cytokinin signalling, sugar signalling, as well as YABBY and MYB-related transcription factors to function downstream of NS1. qRT-PCR analyses of ns1 mutant and 35S:*ns1* overexpression lines confirmed the differential expression of these candidate genes, and implicated additional cytokinin response regulatory genes during NS1 function. Analyses of transgenic plants expressing RFP fusion proteins of the predicted cytokinin response regulator HISTIDINE PHOSPHOTRANSFER PROTEIN (ZmHP1) confirmed its mis-localization in ns1 mutant meristems. These results are consistent with previous *in situ* hybridization analyses of *Zmhp1* transcript, which accumulates in meristematic lateral domains overlapping the expression of *ns1*. Furthermore, *ns1* transcript accumulation is rapidly upregulated by both kinetin and auxin, but is downregulated by the auxin transport inhibitor NPA. Taken together, these data suggest that *ns1* expression and function are feedback-regulated via auxin and cytokinin signaling.

Candidate target genes of WOX3 function were identified using Self-Assembling Autofluorescent Protein (SSAP) microarray technology, in which *Arabidopsis* genomic DNA sequences were bound by immobilized PRS1 protein. Analyses of several interesting candidate target genes are in progress, including *myb3R-4*, the pseudo-response regulator *arr4*, and *wox1*, which functions redundantly with *prs1*.

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P57

Analysis of TOR kinase Pathway Gene Expression in Developing Maize Kernels and Opaque Mutants.

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TOR (Target of Rapamycin) signaling pathway plays essential roles in regulating cell growth in response to nutrients, mitogens, hormones and growth factors, as well as controling anabolic processes such as transcription and translation. Although this pathway is well studied in mammals and yeast, the knowledge of plants is scarce. Maize opaque mutants have increased lysine and tryptophan contents compared to wild type. Previous studies have shown that among several maize opaque mutants including *o1, o2, o5, o9, o11, floury2, defective endosperm* B-30 and *mucronate*, there was increased ribosomal protein gene expression, which indicated that protein synthesis was likely to be altered in these mutants. This result is consistent with a change in TOR signaling, which lead us to investigating the correlation between TOR signaling pathway and the altered protein translation in the mutants. We chose component genes in the pathway that have identified homologues in other organisms to analyze their expression levels during maize kernel development as well as comparisons of *o2* mutant and wild type. Quantitative PCR results showed that during kernel development the expression level of S6 kinase was increased although most of other genes tested were suppressed due to increasing zein transcript abundance. Western blot results confirmed the differences in protein synthesis between *o2* and wild type. Further analysis of TOR signaling pathway genes will increase our understanding of this pathway and its potential role in altering protein translation of *o2* mutants.

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P58

Auxin Evo-Devo: Genetic and genomic approaches to understanding the role of auxin in shoot development

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Auxin regulates almost every aspect of plant growth and development. A better understanding of the role of auxin is fundamentally important to basic plant biology and crop improvement. Previous research has demonstrated both conservation and diversification of the role of auxin in maize and Arabidopsis. This project will further our understanding of how auxin regulates shoot development, with an emphasis on maize shoot organogenesis.

To identify additional genes functioning in auxin-mediated organogenesis, we are characterizing 138 maize mutants with characteristic defects in vegetative and reproductive development. Together with previously characterized mutants, 20 loci have been identified and 9 genes cloned (2 in the past year). The genes underlying 11 loci are currently being identified by positional cloning, and mapping populations are being constructed for the remaining 65 mutants. Many of these genes that have been cloned encode proteins required for auxin biosynthesis, transport and response. Preliminary phylogenetic analysis of these gene families has illustrated complex relationships amongst monocot and eudicot clades.

Further phylogenetic characterization of all identified gene families, in combination with comparative expression analyses, will test the conservation and diversification of the mechanisms of auxin action in all flowering plants. Determination of the molecular, cellular, biochemical and genetic interactions between components in the pathway will provide the detailed information necessary to begin assembling a network of gene interactions. To identify additional genes, we are using a novel method for transient local auxin induction to perform comparative expression profiling in both maize and Arabidopsis. Statistical and mathematical modeling will integrate the expression profiling and interaction data to construct a gene regulatory network, which will provide hypotheses for future experimentation. Together, these interdisciplinary approaches will expand our basic knowledge of plant development and contribute to ongoing efforts to improve maize and other crops for use as both food and fuel.

www.AuxinEvoDevo.org

Funding acknowledgement: National Science Foundation (NSF)

P59

Changes in Mitochondrial Genomes Associated with Reversions of S-Type Cytoplasmic Male Sterility in Maize

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S-type cytoplasmic male sterility (CMS-S) is characterized by high levels of a 1.6-kb mitochondrial RNA carrying unique, adjacent open reading frames, *orf355* and *orf77*. In the S mitochondrial genome, these ORFs are preceded by a sequence that is nearly identical to the terminal inverted repeats (TIRs) of two linear plasmids, S1 and S2, which are also characteristic of CMS-S mitochondria. Homologous recombination between the TIRs of the plasmids and the TIR sequence of the CMS-S-associated region results in linear ends of the mitochondrial genome from which the 1.6-kb RNA is transcribed. Spontaneous reversions to fertility are found in specific nuclear backgrounds. Although revertants in some nuclear backgrounds lose the S plasmids, revertants in the Wf9 nuclear background retain the plasmids and show rearrangements involving the CMS-S-associated region. Some of these events result in partial or full deletions of *orf355-orf77*. We describe cases in which rearrangements have separated an intact *orf355-orf77* from its adjacent TIR sequence. When the rearranged *orf355-orf77* region is expressed from promoters elsewhere in the mitochondrial genome, the plants are male fertile. These observations support the hypothesis that a TIR-localized plasmid promoter is used to drive the synthesis of the CMS-S-associated 1.6 kb-RNA.

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P60

Characterization of the correlation between ZmSERK gene expression and embryogenesis in maize culture

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SOMATIC EMBRYOGENSIS RECEPTOR KINASE (SERK) gene has been linked to somatic embryogenesis in numerous species. The ZmSERK3 gene was firstly identified and characterized at the protein sequence level. A culture model was developed to check the correlation between embryogenesis and ZmSERKs expression profile under different culture conditions. Also the influence of developmental stage on embryogenesis was explored. The data presented that the ZmSERKs expression could be detectable within all the culture period. Auxin 2, 4-D (2, 4-Dichlorophenoxyacetic acid) could enhance the transcription activity of ZmSERKs and promote the somatic embryogenesis, especially ZmSERK1 and ZmSERK3. Cytokinin BA (N6-benzyleadenine) alone inhibits the embryogenesis as well as diminishes the expression of ZmSERK1 and ZmSERK2. However, the expression of ZmSERK3 increased significantly by BA treatment. The application of 2, 4-D plus BA could promotes the transcription of ZmSERK3 and inhibits that of ZmSERK1 and ZmSERK2 suggesting the fine modulation of SERK in embryogenesis. The immature embryo of 12 and 15 day after pollination conferred the highest competence for embryogenesis. These data led us to conclude that the expression profile of ZmSERKs is in close correlation with somatic embryogenesis associated with phytohormone signalling and develomental stage. ZmSERK1 and ZmSERK3 are possibly important to confer the capacity to initiate and maintain the embryogenesis while ZmSERK3 maybe also play a role in differenciation. ZmSERK2 probably acts together in iniciating embryogenesis and also functions in other developmental process exhibited by its expression profile.

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P61

Cold and drought stress induce different cellular and molecular growth responses in maize leaves

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Low temperatures and limited water availability are the two most important factors leading to reduced maize yield due to growth retardation. To study the cellular effects of these two environmental factors, we quantified leaf growth of maize B73 seedlings at low night temperature (4°C) and at limited water supply (soil water potential of approximately -1 MPa).

Although both treatments resulted in a 25% reduction of the steady-state leaf elongation rates, kinematic analysis revealed strikingly different responses at the cellular level: low night temperature reduced the production of meristematic cells by slowing down the cell cycle and had no effect on cell mature cell size. Drought stress in contrast had no effect on cell division rates, but reduced the size of the meristem and affected cell expansion resulting in a reduced mature cell size. These results show that these stresses induce a similar macroscopic phenotype through contrasting effects on cell division and cell expansion.

To investigate this complex interplay between cell division and expansion at a molecular level, we profiled the transcripts of dividing, elongating and mature cells of stressed and non-stressed leaves by micro-array experiments. Moreover, we complemented the transcript profiles with metabolomics data. Based on these results we aim to construct regulatory networks that regulate the growth process and the responses to these agronomically important stress conditions.

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P62

Culture of isolated embryo sacs and caryopses as a biotechnological system for the upgrowing of maize zygotic embryos in vitro for genetic transformation procedures

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Technique of in vitro development of isolated plant female generative structures from early stages to maturity is little processing, but perspective experimental system for modeling the development and maturation of embryo sac, embryo, endosperm, seeds, for recovering key stages and limiting factors in formation of reproductive organs, which for many agricultural crops generate yield and seed productivity. The development of different isolated generative structures grown in vitro up to maturity is an important step in genetic transformation procedures with egg cells, sperms, zygotes, young embryos, in manipulations with single cells, in transferring organelles to gametes and zygotes.

The investigation established the possibility of updevelopment of maize embryo (53 %) in culture of isolated embryo sacs in in vitro conditions at the 7th day after pollination with genotype DK2/477-322xA22 from the transition stage up to the beginning of organogenesis. Embryo sacs were cultured on artificial nutrient medium NBM (Mól, R et al., Planta 189: 213-217, 1993) with 90 g/l sucrose and 2 mg/l 6-benzylaminopurine. It was determined that the cultivation of embryo sacs with the aim to upgrow an embryo and to accumulate starch in endosperm should be continue more than 20 days and nights. The colour of cultured embryo sac can be considered a marker of embryo upgrowing and starch accumulation in endosperm.

Experimental data confirm the possibility of embryo developments (12 %) in culture of isolated maize caryopses at the 5th – 7th day after pollination and represent the production of viable embryos (65 %) germinating in regularly developed green plantlets. Caryopses were cultured on artificial nutrient medium NBM (Mól, R et al., Planta 189: 213-217, 1993) with 90 g/l sucrose and 1 mg/l 6-benzylaminopurine. Isolated caryopses were taken from field donor plants of maize inbred DK366 and hybrid A22xDK307, DK 2/477-322xA22. Genotype significantly affected both growth and development of caryopses in culture, especially starch accumulation in endosperm and covers and a frequency of developed embryos. The internal state of cultured caryopses can be considered a marker of embryo upgrowing and starch accumulation in endosperm independently of genotype. Embryo development and caryopses growth were finished independently of genotype by the 20th day in culture. Soon after this term embryos should be isolated out of caryopses for germination and obtaining plantlets.

P63

Dissecting Homologous Pairing Using the SegII Mutant

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Although pairing of homologous chromosomes is vital for the successful completion of meiosis, the nature of the mechanisms controlling the chromosome homology search and pairing remains poorly understood. Research in several species has identified that double strand breaks (DSBs), which form in chromosomal DNA in early meiosis and initiate meiotic recombination, also play a critical role in homology recognition. In maize, more than 500 DSBs are present in early meiotic prophase, despite the fact that only 20 are required for crossing-over (one per chromosome arm). We found that the segII mutant in maize, which is severely defective in homologous chromosome pairing, sheds light on the role of the DSBs that do not lead to crossover formation. segII exhibits severely reduced numbers of meiotic DSBs, most likely as a result of a mutation in the regulatory region of Spo11, the gene responsible for DSB creation. The reduction of DSB numbers is followed by a reduction of the frequency of homologously paired chromosomes, which, instead, form associations with non-homologous partners. Crossovers are also decreased in number and often form between non-homologous chromosomes. However, interestingly, decreases in the frequency of homologous chromosome pairing (~ 2-fold) and crossing-over (~2 to 4-fold) are not as severe as the decrease in the number of meiotic DSBs (~50-fold)

Our observations suggest that (i) crossovers in maize are being maintained at the expense of non-crossovers under limiting DSB conditions (indicating “crossover homeostasis”), (ii) proper pairing interactions are maintained despite the limited number of sites at which pairing can occur (2% DSB formation) (implying “pairing site homeostasis”), (iii) the additional DSBs beyond the minimum 20 are required for the homology search mechanism(s) to correctly pair all chromosomes with their partners. These DSBs provide additional 'pairing sites' likely allow chromosomes to ignore micro-homology between repetitive elements in favor of true homology between single copy regions.

Funding acknowledgement:

P64

Elucidating the Role of the Extracellular Peptide ZmEBP1 for Embryo Patterning

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Embryo patterning in Arabidopsis strongly depends on auxin gradients and thus the activity of proteins involved in its transport as well as various downstream response regulators including a large number of transcription factors. Homologous genes have been found in maize and basic patterning mechanisms seem conserved. However, it is completely unclear how embryonic cells communicate with each other in order to establish, for example, stem cells and vascular tissue and how tissue boundaries are defined. With the goal to study the function of extracellular candidate signalling proteins expressed during embryogenesis in maize, we are analysing the function of EA1-box proteins. ZmEBP1, for example, encodes a small secreted protein precursor of 74 amino acids. Gen expression is restricted to the developing female gametophyte (FG) and the early stages of embryo genesis. Expression starts after cellularization in the FG and persists until the transitions stage embryo. The finding that activity of the *ZmEBP1* promoter is visible throughout the embryo, but signals of ZmEBP1-GFP-fusion protein is restricted to the ZmPIN1 localized region suggests that ZmEBP1 is strongly regulated at the post-transcriptional level. In order to analyse its biological function, RNAi knock-down phenotypes are analysed, a bioassay using recombinant protein is established interaction partners of ZmEBP1 in the developing maize embryo will be isolated allowing us to describe its role and to gain new insights into the fundamental processes of pattern formation and cell fate determination in early embryo development.

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P65

Embryos desiccation tolerance in maize viviparous mutants

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The maize embryo acquires desiccation tolerance during maturation stage, around 25 days after pollination (DAP). During this period abscisic acid (ABA) inhibits precocious germination therefore mutants impaired either in ABA biosynthesis or sensivity, are viviparous. To assay if ABA or other molecules have a function in the process that leads to acquisition of dehydration tolerance, we compared the germination of immature (25 DAP) embryos of viviparous and wild type sibs, prior to or after induction of an artificial dehydration treatment. The results show that all albino mutants impaired in the early stages of ABA biosynthesis have lost desiccation tolerance, whereas mutants impaired in the late stages (green seedlings) as well as *vp1* (a response mutant) exhibit a partial tolerance. These results suggest that ABA itself or other molecules in ABA pathway like carotenoids could be the candidates molecules involved in the protection of the embryo from dehydration damage. We further analyzed *vp10-105*, a green mutant impaired in ABA biosynthesis showing high rate of water loss, transpiration and stomatal conductance. Genes that normally are expressed in water stress condition, seem expressed independently of the water regime suggesting that the mutant is constitutively water stressed.

P66

Evolution of RAMOSA3-like genes in grasses (Poaceae)

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The three *RAMOSA* genes in maize, *RAMOSA1* (*RA1*), *RA2* and *RA3* are essential for the formation of short branch (or spikelet pair) meristems, with *RA2* and *RA3* acting as upstream regulators of Maize has two *RA3-like* genes – *ZmRA3* and *SISTER OF RA3* (*ZmSRA*) on chromosome 7, whereas the syntenic regions in rice and brachypodium have only a single gene that is most similar to *ZmSRA*.Phylogenetic analyses estimate that the *RA3/SRA* duplication occurred prior to major diversification of grasses with *RA3* orthologs estimated to have been subsequently lost near the base of the rice, wheat and bamboo lineage (BEP clade). *ZmRA3* expression is restricted to a cup-shaped region subtending the spikelet pair meristems and a stripe between the upper and lower florets, whereas *ZmSRA* is expressed broadly throughout the plant with the highest expression in roots. Expression analyses of *SRA* and *RA3* in other grasses reveal a complex pattern of evolution. *SRA* orthologs are expressed at the base of long branches in rice, barley, and sorghum and between the upper and lower florets and the pistil in teosinte. *RA3* expression was detected subtending long and short branch meristems and between the upper and lower florets in sorghum and teosinte and on the adaxial surface of florets in chasmanthium. Together these data suggest the restricted pattern of *RA3* expression detected in maize subtending short branch meristems did not coincide with the evolution of these structures within panicoid grasses, but appears to have evolved recently, potentially during the domestication of maize.

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P67

Expression and localization of ZmRAB2A and ZmRAB1A in the developing maize leaf

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Cells in a maize leaf grow in an orderly pattern that contributes to the final shape of the leaf. We are studying proteins that keep order in the leaf by maintaining the gradient of cell expansion. When the gradient is lost, cells become overly expanded and cause a wart-like phenotype. We identified the role of ZmRAB2 in this process through associating the wart-like phenotype with insertions of Mu into ZmRAB2A. To understand the role of RAB2A, we have studied its expression and sub cellular localization along the leaf gradient in relation to its potential partner in vesicle trafficking, ZmRAB1A. Both RAB1A and RAB2A function together in the ER-Golgi compartment in yeast and mammalian systems and we are determining if the plant homologs function similarly. Results from RT-PCR along the developing leaf gradient show that ZmRAB1A and RAB2A are expressed in overlapping domains at the leaf base: ZmRAB1A is restricted to the basal most dividing domain, whereas ZmRAB2A extends in a gradually decreasing gradient of expression to the leaf tip. Thus, any shared function is not clearly restricted to cell expansion or alternatively, the site of exclusion of ZmRAB1A may represent a distinct domain of expansion in the developing leaf. To examine protein distribution, we tagged each with a distinct fluorescent protein to test ultimately for co-localization in plants harboring both fusion proteins. We are using live cell imaging of ZmRAB2A-YFP and ZmRAB1A-CFP to compare subcellular location and we report the localization pattern of each FP line here. Both show similar punctate localization in small rapidly growing cells, with rapid protein movement, that is both perinuclear and distributed through the cytoplasm, in patterns consistent with RT-PCR results. Immunolocalization and gradient centrifugation supports partial co-localization between ZmRAB2A and ER. The double fusion line is currently being generated to identify the spatial distribution of the proteins, particularly at their overlap zone in the developing leaf.

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P68

Expression of the Maize Cellulose Synthase (CesA) Gene Family at the Cell-, Protoplast-, and Tissue- Levels.

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Our overall goal is to understand regulation of genes that control synthesis and integration of the diverse components of plant cell walls. For maize in particular, this will aid collective efforts to improve its use for grain, fiber, and renewable energy. Here, we use gene-specific qRT PCR to profile expression of the cellulose synthase (*CesA*) family at cell- and tissue- levels. Tissue-level expression was analyzed during three phases of development; seedling emergence, vegetative growth, and anthesis. Data allowed grouping of *CesA* family members based on overall similarities in expression patterns across tissues. Three main categories emerged; highly similar expression (*CesA10,11,12*), similar expression with slight variations (*CesA1,2,6,7,7-paralog,8,9*), and independent expression (*CesA3,4,5*). As development progressed, expression patterns became less variable. The *CesA10,11,12* group was primarily expressed in "woody" tissues (stem, prop root and husk), consistent with earlier work demonstrating an association of these genes with arabidopsis *CesA's* proven to be specifically involved in secondary cell wall synthesis. In addition, a protoplast system was used to examine *CesA* expression throughout cell-wall regeneration. During the first 60 hrs of this process, mRNA levels of *CesA* family members rose 3.5 to 35 fold. Upregulation of *CesA's* was not evident until 12hrs of incubation, consistent with the initial deposition of callose is as the main cell wall constituent deposited. When less sucrose was supplied during cell wall regeneration, the timing and extent of *CesA* upregulation shifted for different members of the *CesA* family. Collectively, the profiles indicate that expression of the *CesA* gene family is dynamic at both the cell- and tissue- levels. This is concurs with previous hypotheses that different CESA's may function in diverse heterohexameric complexes and are highly regulated.

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P69

Expression of the Maize Chromatin Assembly Factor-1 Genes

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Chromatin assembly factor-1 (CAF-1) is a heterotrimeric protein complex involved in DNA replication-associated chromatin assembly. CAF-1 is essential for proper development in plants. *ZmFas1*, *ZmFas2* and *ZmMSI1* encode the three subunits of CAF-1 and are all expressed at higher levels in shoot apical meristem as compared to whole 14-day seedling. In *Zea mays* there are two *Fas1* paralogs designated *ZmFas1a* and *ZmFas1b*, which encode proteins with approximately 90% amino acid similarity. As anticipated from the Arabidopsis *Fas1* sequence, the *ZmFas1* paralogs each have two putative E2F binding sites within their promoter regions. Within the B73 genome *ZmFas2* appears to be present as a single copy, while *ZmMSI1* is a member of a small gene family. RT-PCR analysis of multiple tissues from B73 plants indicates that both *ZmFas1* paralogs are expressed to a similar level in all tissues examined; tissues that are mitotically active exhibit a high level of expression, consistent with CAF-1 molecular function. *ZmFas2* and *ZmMSI1* are similarly expressed in these tissues. RT-PCR analyses of seedlings from 11 North American inbred lines, reciprocal hybrids of B73 and Mo17, and 5 open-pollinated Mexican land races demonstrated variable *ZmFas1* paralog expression, with *ZmFas1b* generally being expressed at a higher level. Quantitative RT-PCR analyses of *ZmFas1* paralogs confirm these observations.

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P70

Female Gametophyte Cell Identity in Maize is Regulated by diSUMO-Like (ZmDSUL) and EA1-Box Protein ZmEBP1

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An excellent, but challenging model system to study the establishment and maintenance of cell identity in plants represents the formation of the haploid female gametophyte (FG). After female meiosis (megasporogenesis), three spores degenerate and the functional megaspore develops after three rounds of free nuclear divisions into the immature female gametophyte consisting of eight nuclei. After nuclei movement and positioning, seven cells are formed consisting of two female gametes, egg and central cell, that produce embryo and endosperm after fertilization, two synergids involved in pollen tube attraction and sperm delivery as well as antipodal cells of unknown function. This process is called megagametogenesis. In order to obtain a clue about the molecular mechanism(s) regulating cell identity, we have characterized the role of genes exclusively expressed in egg cells during female gametogenesis. Here we will report about the role of the diSUMO-like protein ZmDSUL that contains two head-to-tail SUMO-like domains and the auxin induced egg cell secreted EA1-box containing peptide ZmEBP1. Unter control of their endogenous promoters, ZmDSUL-GFP and ZmEBP1 fusion proteins were first detectable in the micropylar-most position of the female gametophyte at stage FG 5/6, when migration of polar nuclei and cellularization occurs. Mature FGs displayed GFP signals exclusively in the egg cell. RNAi silencing showed that both genes are required for FG viability. However, while nuclei segregation and positioning defects occurred at stage FG 5 in *dsul* mutants, *ebp1* mutants showed mis-specification of antipodal cells. The non cell-autonomous function of ZmEBP1 is currently investigated using recombinant protein.

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P71

Functional characterization of RUM1 protein-protein interactions

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Plant roots play important roles in water and nutrient uptake, and anchorage of the plant in the soil. Maize (*Zea mays* L.) roots consist of the embryonic primary root and seminal roots, as well as postembryonic lateral and shoot-borne roots. The monogenic mutant *rum1* (*rootless with undetectable meristem 1*) of maize (*Zea mays* L.) is deficient in the initiation of the embryonic seminal roots and postembryonic lateral roots at the primary root (Woll *et al.*, 2005). Cloning of *RUM1* indicated that it encodes for an AUX/IAA protein. Because of a 1.7 kb non-*Mu* transposon insertion in exon 2, which leads to alternative splicing, the mutated rum1 protein shows a 24 AA deletion in the AUX/IAA domain II.

AUX/IAA proteins interact with ARF (auxin response factor) transcription factors which regulate root development in *Arabidopsis thaliana* (Fukaki *et al.*, 2002). In order to better understand the molecular interactions during RUM1 dependant lateral root formation, interaction partners of RUM1 were identified via a yeast two hybrid screen. Moreover, GST-pull down experiments and in vivo split-YPF, indicate that RUM1 interacts with the closely related AUX/IAA protein RUL1 (RUM1-like protein), and also forms RUM1/RUM1 homodimers.

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P72

Genetic Background Effects on Phenotypic and Genotypic Expression of *Cg1*

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Phase change, or the transition from one distinct form of development to another, is essential to the lifecycle of higher plants, which undergo these transitions at the shoot apex. The *Corngrass1* (*Cg1*), mutation of maize is a heterochronic mutation that prolongs the juvenile-vegetative phase. This mutation, when highly expressed, results in short internodes, multiple tillers, slender culms and leaves, a bluish, epicuticular wax on the entire plant, reduced ear size, and the conversion of the tassel to a vegetative structure. The *Cg1* mutant is caused by a *STONER* retrotransposon insertion that drives ectopic expression of miR156. There is evidence that vegetative phase change is regulated by the relative levels of miR156 and miR172. Mo17 has been reported to suppress the *Cg1* phenotype relative to other maize inbreds such as B73. The first objective of this work was to quantify effects of the B73 and Mo17 genetic backgrounds on *Cg1* phenotypic expression by phenotyping typical *Cg1* traits among B73, B73*Cg1*, Mo17, and Mo17*Cg1* crosses in a nested mating design. The second objective was to test whether B73 and Mo17 genetic backgrounds caused differences in miR156 and miR172 expression with real-time RT PCR analysis using the same nested mating design. Both experiments showed significant genetic background effects. B73 had significantly greater expression of seven of the eight measured *Cg1* phenotypic traits as both the maternal and paternal parent relative to Mo17. B73 also had significantly greater expression of miR156 relative to Mo17 in the presence of *Cg1*. There is also evidence for heritability of the degree of Cg1 expression across and within inbred backgrounds. When combined, results of the two experiments show a relationship between miRNA expression and the *Cg1* phenotype.

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P73

Genetic analysis of the trans-acting siRNA (ta-siRNA) pathway and adaxial-abaxial patterning in maize

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The *leafbladeless1* (*lbl1*) mutant displays a defect in leaf polarity such that the adaxial (upper) surface of the leaf often displays regions of abaxial (lower leaf surface) identity. This loss of adaxial identity coincides with ectopic blade outgrowth, and in more severe cases, entire leaves become abaxialized and exhibit a radial phenoype. *lbl1* encodes the homolog of *Arabidopsis* SUPPRESSOR OF GENE SILENCING3 (SGS3) and is required for the biogenesis of 21 nucleotide transacting small interfering RNAs (ta-siRNAs) that mediate the post-transcriptional repression of *auxin response factors 3* (*arf3*). Mutations affecting other genes in the ta-siRNA pathway have been identified, including *ragged seedling2* (*ago7*), *RNA-dependent RNA polymerase 6* (*rdr6*) and *dicer-like 4* (*dcl4*). Null alleles of *lbl1* and *rdr6* result in embryos with a shoot meristem-less phenotype, however null alleles of *dcl4* produce a phenotype that resembles that of the weak *lbl1-ref* allele. A hierarchical function for the four DICER-LIKE family members has been demonstrated in *Arabidopsis*. To test this possibility, we are characterizing mutations in other members of the maize dicer family. Using this collection, as well as other mutants, we are further investigating the ta-siRNA pathway in maize and its role in meristem function and leaf development.

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P74

Genetic network regulating development of embryo and lateral organs by *Viviparous8* and *Big embryo1*

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Plant architecture impacts yield and biomass productivity. For instance, leaf number is a critical component of light harvesting area, and branching of the root system affects water and nutrient uptake. The architecture of leaf and root is determined by complex genetic interactions that regulate cell division, growth and differentiation. In maize the *Viviparous8* (*Vp8*) and *Big embryo1* (*Be1*) genes have key roles in determining the number of leaves and adventitious roots produced by the plant. In contrast to other genes implicated in lateral organ formation, these two loci also regulate embryo development. Whereas mutations in the *Vp8* locus cause severe defects in embryo development in W22 inbred, the *be1* mutants show pleiotropic but relatively more specific and subtle changes in plant development including formation of viable embryos. As reported previously, the *be1* mutants develop embryos with significant enlargement in the scutellum. The adult mutant plants develop additional leaves. Inspection on the developing embryos of wild type and *be1* mutant revealed that the cell size of scutellum is profoundly larger in the mutant embryos. Interestingly, lateral root primodia develop ectopically in the premature embryo of *be1* mutant. Consistent with the ectopic formation of lateral root primodia in developing *be1* embryos, mutant plants develop additional seminal roots as well as aerial roots. In addition to the embryonic root phenotype, the size of embryonic shoot meristem is approximately one-third smaller in the mutant embryo than the wild type. We examined expression of LEC1 and B3 domain transcription factors such as *Vp1* in developing *be1* embryos. Among these master regulators for seed maturation, expression of a subset of *LEC1* genes is specifically altered in the *be1* developing embryos suggesting that *Be1* functions independently of *Vp8* in regulation of the seed B3 genes. However, the double mutant analysis of *be1* with ABA deficient *vp14* and ABA insensitive *vp1* respectively shows positive genetic interactions between these regulators of seed maturation. These results suggest that *Be1* has a key role in an as yet identified regulatory step by interacting with an ABA- and B3 factor-mediated pathway for embryo development.

Funding acknowledgement: United States Department of Agriculture (USDA)

P75

Genome-wide identification of KNOTTED1 targets using ChIP-Seq.

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KNOTTED1 (KN1) and other class I homeobox (KNOX) transcription factors are involved in the establishment and maintenance of plant meristems. They act in part through the modulation of hormones such as cytokinin and gibberellin (GA), but they also negatively regulate secondary cell wall synthesis. However, few direct KNOX targets have been recognized so far. In maize, the use of a candidate gene approach in combination with chromatin immunoprecipitation (ChIP) led to the discovery of the KN1 target ga2ox1, which codes for an enzyme that inactivates GA. To identify the targets of KN1 on a genome-wide level, we used high-throughput sequencing of ChIP DNA (ChIP-Seq). This technique, applied to two biological replicates, has allowed us to establish a list of around 200 loci occupied by KN1 in immature tassels. Most of the loci are located in the vicinity of genes models and a significant proportion is specifically located within introns, similar to the KN1 binding site in the ga2ox1 gene. Manual validation by quantitative PCR of a subset of the loci confirmed the binding in immature tassels but also in immature ears and in the shoot apical meristem. Quantitative RT-PCR confirmed that some of the genes are modulated in a loss-of-function kn1 mutant background. Overall, our results indicate that our list of KN1 binding loci identified by ChIP-Seq is trustworthy, despite that only a fraction of the associated genes can be clearly linked to the gain- and loss-of-function mutant phenotypes.

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P76

Hormonal and temperature regulation of far-red light signaling in the maize seedling.

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In dense plant stands, the selective absorption of red (R) and blue light by photosynthetic pigments causes a reduction in the ratio of R to far-red (FR) light. The phytochrome photoreceptors can perceive R:FR and induce a cascade of morphological and physiological adaptations referred to as shade avoidance syndrome (SAS). Traits that are affected include leaf angle, flowering time and stem elongation. To study this phenomenon in maize, a crop for which high density is paramount to maximize yield, an end-of-day-FR (EOD-FR) assay was developed to study response of plants to FR in seedlings. We examine the roles of GA and ABA in mediating EOD-FR responses and have used phytochrome deficient elm1 and phyB single and double mutants (introgressed into two inbred lines) to genetically dissect this response. Pharmacological treatments using GA3 or paclobutrazol in combination with EOD-FR revealed a tissue-specific control of the seedling elongation and suggest a rate-limiting role for GA in mediating mesocotyl elongation. The constitutive EOD-FR responses of the elm1 and phyB1 phyB2 double mutants were greatly attenuated when a chilling temperature was applied during night breaks. These results suggest a temperature-dependent role for the different phytochrome members in mediating EOD-FR response.

P77

Insight into density tolerance – is plant to plant variability the key to density tolerance?

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Genetic improvement studies in maize have given breeders and geneticists valuable insights into the mechanisms underlying grain yield. These changes along with agronomic improvements have resulted in a 7-fold increase in grain yield in 7 decades of intensive breeding efforts (e.g., Duvick 1997, 2004; Lee and Tollenaar, 2007). From these types of studies we know that improvements in stay-green and density tolerance have made the largest contributions. Plant population density has doubled in the last 7 decades, with most modern hybrids being grown at 80.000 plants/ha. At a biological level, what makes one genotype more tolerant to higher plant densities than another genotype? The dynamics of dry matter accumulation and partitioning of the dry matter to the ear seem to only partially explain density tolerance. Most plant density research has focused on treating the stand of plants in a field as a single unit, rather than as a population of plants. Plant-to-plant variation exists in every maize field, however, this variability increases when plants are grown beyond their optimum densities, and plant competition for resources (e.g., light, water and nutrients) increases. Is there a corresponding improvement in plant-to-plant variability and can this possibly explain density tolerance? Using two contrasting year of release hybrids, Pride 5 (released in 1959) and Pioneer 38N87 (released in 2007) variability in the relationships between: i) kernel number and plant growth rate around silking and ii) grain yield and dry matter at maturity were examined.

P78

Poster Removed

P79

Investigation of molecular mechanisms controlling determinacy of the spikelet-pair meristem.

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The characterization of molecular mechanisms controlling architecture of cereal inflorescences, and the identification of novel genes that participate in this process are crucial for understanding the development of grass inflorescences, and may be used to improve yield traits of commercial crops. In current research, we use maize mutants, ramosa1 (ra1), ramosa2 (ra2) and ramosa3 (ra3) that show indeterminate spikelet-pair meristem phenotype, in order to characterize molecular networks regulated by these genes. Several approaches are applied for this purpose. First, we use ILLUMINA/ SOLEXA transcriptome analysis to identify the genes regulated by RAMOSA gene activity. Second, we perform ramosa3 enhancer/suppressor screen to identify the genes that participate in the network. Third, we develop novel approaches for the imaging of fluorescent-labeled molecular markers that are introduced into the mutants' background. Fourth, we create double-mutant combinations of the ramosa genes with maize meristem maintenance genes, such as knotted1 (kn1), fasciated ear2 (fea2), and thick tassel dwarf1 (td1), and with the genes involved in auxin transport and synthesis, such as barren inflorescence2 (bif2) and sparse inflorescence1 (spi1). Combining these approaches will enable us to better characterize the mechanisms of the RAMOSA gene activity, and the activity of genes that participate in the RAMOSA network.

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P80

Isolation of a RNA-binding protein gene from a collection of *Mutator*-induced seed mutants

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In maize, the size, shape and content of the two seed compartments (embryo and endosperm) is determined during seed development, a process involving an estimated 1000 genes, of which less than 10% have been cloned and analyzed so far. The target of our project is the isolation and functional characterization of genes involved in maize kernel development by means of molecular analysis of transposon-induced seed mutants. The mutants were developed by Biogemma SAS and show a clean 3:1 segregation of defective kernel phenotype. For mutant screening, a novel cDNA-based transposon display technique, which was developed within the Muexpress ERA-Net plant biology project, was used.

One transposon flanking sequence encoding a mitochondrial-targeted RNA-binding protein (ZmRBP) showed 100% linkage to the phenotype. Histological studies of the mutant kernels revealed an obvious retarded embryo growth, significant starch reduction in central starchy endosperm and irregularly shaped cells in the basal endosperm transfer layer. Similarity search identified a single ZmRBP-homologous gene in *Arabidopsis:* AtRBP. Genetic complementation by overexpression of ZmRBP in *Arabidopsis Atrbp* T-DNA knockout plants fully complemented the ZmRBP-related mutant phenotype confirming the causality of the gene. GFP/dsRed fusion studies showed a subcellular localization of ZmRBP in the mitochondria. To identify the target mtRNA associated *in vivo* with ZmRBP a co-immunoprecipitation coupled with RT-PCR was carried out. This assay revealed a potential association between ZmRBP and the ribosomal protein subunit 3 (rps3) mRNA. *In vitro* analysis by Electrophoretic Mobility Shift Assay confirmed the binding of ZmRBP to rps3.

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P81

Kernel number per ear: A role for sorbitol dehydrogenase

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In maize, sorbitol biosynthesis is mediated primarily by sorbitol dehydrogenase (SDH) (Sorbitol + NAD fructose + NADH), and previous data indicate that the sdh1 mutant phenotype has smaller and more abundant kernels per ear. Work here further tested field responses of mutant and wild-type siblings from nine genotyped families (385 individuals), using material that had been backcrossed 4 times into W22. The initial sdh1 mutant had been isolated from the Uniform Mu population (which has a uniform W22 background), but additional backcrosses were employed to further test the relationship between mutant and phenotype. Seeds from segregating F2 ears were grown in fall 2009, using a staggered field design with each family in at least two sites and planted at three intervals (7 to10 days).. All plants were genotyped and selfed. Little to no difference was evident in field emergence of sdh1 and wild type siblings, regardless of planting date or field location. Capacity for plants to produce an ear was also similar. However seed number per ear was consistently greater for sdh1 mutant, with an average increase of 80%. Previous work (prior to backcrossing) also indicated a similar increase in kernel number per ear. In all analyses thus far, the extent of seed size reduction (20%) was more than compensated by the increased number, thus resulting in consistently greater total dried seed weight.

Introgression of the sdh1 mutant into diverse inbred (B73, Mo17, B104, HyII) and sweet corn lines (sh2 and su1) are in progress to further test effects of the sdh1 mutation. The sweet corn lines are of particular interest due to earlier work that showed increased sugar levels in the sdh1mutant (2 fold more hexoses and 16% more sucrose) at 20-25 days after pollination. The increased hexoses may indicate a central role of SDH in sorbitol metabolism and sugar balance of developing maize kernels.

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P82

Maize SUN Domain Proteins; A 5-Member Gene Family Encodes Two Distinct Classes (CCT and PM3 types) of Putative Nuclear Envelope Proteins.

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The nuclear envelope (NE) provides a physical barrier which separates the contents of the nucleus from that of the cytoplasm. The NE consists an inner nuclear membrane and the outer nuclear membrane, separated by the perinuclear space. Relatively little is known about the proteins that reside and function in the plant NE, despite the expectation that they are conserved amongst eukaryotes. Essential NE functions include a role in meiotic telomere clustering, nuclear position and motility within the cell, and cell-cycle-dependent breakdown and reformation. Recent work in non-plant systems has identified a class of NE proteins that function to link the nucleus to the cytoplasmic motility systems. These NE proteins share a SUN (Sad1p/Unc-84) homology domain and are widely conserved among eukaryotes. We initiated a search for SUN domain proteins in maize using animal and yeast SUN protein sequences to query plant EST and genome databases. We found five different unlinked SUN domain-containing genes (*ZmSUN1-5*) located throughout the genome. These fall into two structural classes based on protein secondary structure and phylogenetic analysis. One of these resemble animal and fungal SUN domain proteins in that they all have a C-terminal SUN domain. We call these (*ZmSUN1* and *ZmSUN2*) canonical C-terminal type (**CCT**). In addition, we discovered a novel class characterized by the presence of a SUN domain in the middle of the protein and three transmembrane domains. We call these (*ZmSUN3*, *ZmSUN4*, *ZmSUN5*) **PM3** type. The *ZmSUN3* gene was identified as a candiate gene for *desynaptic* (*dy*) in previous mapping studies. Peptide antibodies against SUN3 showed NE staining and detected a band of approximately 70 KDa on a western blot. Current studies are underway to further characterize the novel plant-specific PM3 type of SUN proteins and define their topological arrangement within the NE.

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P83

Maize Stem Parenchyma – Developing Tools for the Engineering of a Potential Storage Tissue

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In some members of the *Poaceae* soluble carbohydrates are stored within stem parenchyma tissue. Sugarcane, for example stores high levels of sucrose, whereas some wheat varieties are transiently storing fructans in the stem tissue. In the major crop plant maize no substantial accumulation of soluble carbohydrates or other assimilates in the stem is known. To possibly use the maize stem parenchyma tissue for biotechnological applications, like molecular farming, the increase of stem biomass or the accumulation of soluble carbohydrates, the availability of strong and specific promoters is required. In order to gain more information on the function and importance of the maize stem parenchyma we chose an approach of next generation sequencing to gather transcriptome data for this particular tissue. With these data we screen for strong and specifically expressed genes. It is planned to isolate promoters from such genes and to evaluate them by promoter-reporter analysis in transgenic maize plants. These promoters will provide valuable tools for the biotechnological engineering of the stem parenchyma as a potential storage tissue. Additionally the analysis of the expressed genes will give insights into the biological function of the maize stem parenchyma.

P84

Meristem determinacy: ear versus leaf

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Plant meristems drive the generation of new tissues and organs and are therefore at the basis of crop productivity. In maize, the vegetative leaf primordial meristem and female inflorescence immature meristem emerge from the SAM. Both meristems initially generate cells in a seemingly indeterminate manner. At a given point however they become determinate and switch off. Since the number of seeds and biomass per maize plant is directly proportional to the number of formed spikelets and leaf length, this switch of the meristems to their determinate state largely determines the number of spikelet per ear and biomass in vegetative tissues. Therefore, this project aims to examine the cellular and molecular basis of this switch between indeterminate and determinate meristem function and to identify and compare regulatory gene-networks between the two systems. The timing of the developmental switch was pinpointed microscopically for each type of meristem, determining the sampling points for micro-array analysis. The results are superimposed on the normal exit from the meristem during the indeterminate growth phase as occurs along the axis of the leaf, which was previously analyzed in our group by transcriptomics. The data allow a better understanding of the processes involved in meristem determinacy and transition of cells from meristematic state to post-meristematic cell expansion, both key steps in plant growth regulation.

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P85

Molecular interactions of the LOB domain transcription factor RTCS which is involved in shoot-borne root formation of maize

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Plant roots serve important functions among which water and nutrient uptake and anchorage are the most important ones. The complex root system of maize consists of the embryonic primary root and seminal roots, and an extensive postembryonic, shoot-borne root system composed of crown- and brace-roots which make up the major backbone of the root system. Crown roots are formed at consecutive underground stem nodes, while brace roots are formed late in development from aboveground stem nodes.

The monogenic recessive mutant *rtcs (rootless concerning crown and seminal roots)* is impaired in the initiation of shoot-borne roots including crown, brace, and seminal roots (Hetz *et al.*, 1996). Goal of this study is the analysis of the molecular networks involved in shoot-borne root formation in maize. *RTCS* encodes a LOB domain protein (Taramino *et al.*, 2007), which localizes to the nucleus. *RTCS* expression is induced by auxin. Recently, we demonstrated that ARFs (AUXIN RESPONSE FACTORS) bind directly to Auxin Responsive Elements (AuxREs) of the *RTCS* promoter *in vitro*. Moreover, RTCS regulates *ARF* expression via binding to LBD motifs (5'-GCGGCG-3') in the promoter of *ARF* genes, which suggests a regulatory feedback loop. Novel direct target genes of RTCS were identified by microarray analyses.

1. Hetz W., Hochholdinger F., Schwall M., Feix G. (1996) Plant J 10: 845-857
2. Taramino G., Sauer M., Stauffer J., Multani D., Niu X., Sakai H., and Hochholdinger F. (2007) Plant J., 50: 649-659

P86

Multiple archesporial cells 1 (mac1) is required for cell fate determination during anther development

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During anther development, cell division and differentiation lead to the formation of four nonreproductive cell layers and the archesporial cells (meiocytes). In maize, the *multiple archesporial cells 1* (*mac1*) mutant exhibits excess archesporial cells, and the surrounding somatic tapetum layer is not developed. Analyses of the *mac1* mutant showed that excess archesporial cells appear in very early anther development and cell division of the primary parietal layer is abnormal. Cloning of *mac1* gene revealed that it encodes a small protein of 213 amino acids that shares similarity with Arabidopsis *TPD1* (*TAPETUM DETERMINANT1*). MAC1/TPD1 is hypothesized to be an extracellular ligand of a Leu-rich repeat receptor protein kinase. In maize, the *mac1* gene is expressed in roots, anthers and ears, and RNA transcripts and protein are enriched in immature anthers. Immunolocalization of MAC1 demonstrates that it is in all cell types in early anther development and is restricted to meiocytes and tapetal cells at late stages. Our data suggests that MAC1 plays an important role in cell fate determination of archesporial and tapetal cells during anther development.

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P87

New functions for maize CK2 b subunits

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Protein kinase CK2 is a highly pleiotropic Ser/Thr kinase conserved in all eukaryotic organisms. CK2 is a heterotetrameric enzyme composed by two catalytic (CK2a) and two regulatory (CK2b) subunits. CK2b regulatory subunits enhance stability, activity and specificity of the holoenzyme but can also interact and modulate other proteins in absence of CK2a catalytic subunits. Land-plants CK2b regulatory subunits present striking features compared to their animals or yeast counterparts, notably (i) belong to multigenic families (up to four members) and (ii) present an additional specific N-terminal extension of about 90 aminoacids sharing no apparent homology with any previously characterized functional domain. Here, by using maize CK2β-1 regulatory subunit as a model, we have performed phylogenetic studies as well as an exhaustive molecular characterization to examine the origin, evolution and functional traits of CK2 N-terminal domain in plants. Our results suggest independent evolutionary diversification CK2 in plants and the acquisition of the N-terminal domain as encoded by a single exon when they conquered land. We have also demonstrated that CK2b-1 N-terminal domain has an important role in regulation of CK2 activity under certain substrates as Rab17. Moreover, N-terminal domain is able to enhance protein aggregation in nuclear speckles and stabilizes CK2β-1 protein delaying its degradation by proteasome. Lastly, by using bimolecular fluorescence complementation (BiFC) we show the in vivo subcellular localization of maize CK2 holoenzyme, which is different from those of the CK2 subunits alone, suggesting that CK2 regulatory subunits are able to export CK2α from nucleus to cytoplasm.

P88

Novel maize mutants impaired in cell differentiation during anther development

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The main goal of the "The Anther Project" (an NSF sponsored plant genome research project, Cell Fate Acquisition in Maize, PIs: Virginia Walbot, and Zac Cande) is the identification of genes required for setting cell fates prior to meiosis and for the regulation of the switch in the cell cycle from mitosis to meiosis. To identify these genes, we examine male sterile mutants for cytological defects in early anther development. We use EMS mutagenized maize mutants, RescueMu mutants from the Maize Genetic Stock Center and mutants from the Maize-Targeted Mutagenesis (MTM) project. The five cell types in anther locules are analyzed in cross-sections of anthers embedded in Spurr's resin. Of the 121 families screened in 2009, 10 mutants were found to have defects in meiotic prophase and 11 had defects in anther wall morphology. Some mutants showed defects in particular cell layers (additional periclinal divisions) while other mutants fail to differentiate some of the layers or even to form anthers. Premature PMC and/or tapetal layer degradation, cell vacuolization in middle and/or tapetal layers were characteristic of several mutants. Complementation tests showed that five of 20 novel mutants identified in 2007 and 2008 are allelic to known maize mutants (msca1, ms8, ms32, ms45). The most interesting mutants defective in cell fate acquisition or maintenance will be subjected to transcriptome and proteomics profiling of anthers and dissected cell types.

P89

Phenotypic characterization and mapping of a fasciated ear mutant.

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Maize inflorescence architecture, and ultimately crop yield, is determined by the activity of meristems. The size of the stem cell niche in a meristem is precisely controlled through positive and negative feedback loops. This is absolutely critical for function as an imbalance in size homeostasis can result in a fasciated, or enlarged, meristem phenotype. We have isolated a recessive mutant from an EMS mutagenized A619 inbred population with a fasciated ear and thick tassel phenotype, which we call fea1905. Scanning Electron Microscopy (SEM) revealed that the fasciated inflorescence phenotypes are due to an enlarged inflorescence meristem. We created two F2 mapping populations by crossing fea1905 (A619) to the B73 and W23 inbred lines and then selfing the F1 progeny. We mapped the mutation to the long arm of chromosome 6 using a set of 764 SNP markers with genome-wide coverage. Fine mapping was carried out using 528 F2 mutants from the two mapping populations. CAPS markers were used to narrow down the mapping interval to a 6 Mbp region, which contains approximately 80 annotated genes. A putative second allele was found by mapping another fasciated ear mutant to the same region of chromosome 6 by bulked segregant analysis. Molecular cloning of the gene will further our understanding of the pathway controlling inflorescence meristem size in maize. Genetic and molecular analyses will determine how fea1905 interacts with established meristem regulating genes such as FEA2 and TD1.

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P90

Polycomb Genes Controlling Endosperm Development in Rice

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Genes of the Polycomb group family (PcG) have been implicated in the maternal control of endosperm development in the model species, *Arabidopsis thaliana*. Wild type PcG proteins assemble in a chromatic remodeling complex and repress transcriptional activity of target genes. Genetic studies in Arabidopsis have identified four PcG genes, *MEA*, *FIS2*, *FIE*, and *MSI1* that are required for normal endosperm proliferation and patterning. In maize, *FIE* and three orthologs of *MEA*, *Mez1*, *Mez2*, and *Mez3* have been identified. Although no functions are known for these genes in maize, some maize PcG genes are known to be expressed from only one parental allele during endosperm development while the other allele is silenced typically through methylation. We are investigating the potential functions of candidate orthologs of the PcG genes in seed development in rice. Sequence database searches have identified rice homologs of the Arabidopsis *FIS2*, *MSI1*, and maize *Mez* and *FIE2* genes. Preliminary expression analysis demonstrates the expression of several PcG genes in the rice seed before and after fertilization. These data indicate that PcG genes might play an essential role in endosperm patterning in cereals.

Funding acknowledgement: United States Department of Agriculture (USDA)

P91

Possibilities to increase the doubling rates in haploid maize seedlings while reducing their mortality

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The doubled haploid (DH) technique has become an important tool in maize breeding. However, the frequency of generating DH plants needs to be improved. Therefore, the results presented in this study will be focused on methods for optimizing the doubling rate of chromosome sets as well as on improving the viability of DH seedlings.

1. Increasing the doubling rate by colchicine application.
2. Improving the viability after colchicine treatment.
3. Using alternative mitotic inhibitors.

After initiation of germination a suitable time point for *in vivo* application of colchicine was determined. By flow cytometry meristematic tissue of seedlings was evaluated. A high percentage of doubled chromosome sets was observed when colchicine was applied two days after germination. To obtain higher survival-rates of maize seedlings after application of this mitotic inhibitor, several approaches were followed. Originally the seedlings had a high mortality-rate after application of colchicine. The viability of seedlings improved considerably after aeration of the culture medium was implemented. Regular aeration of the inhibitor substrate had positive effects on the viability and also on the doubling rate of chromosomes. After establishing an improved treatment system it was interesting to test other mitotic inhibitors with less toxicity to maize seedlings. Preliminary results were obtained by using APM which is known to have lower toxicity.

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P92

Proteomic and genetic dissection of root hair formation in maize (*Zea mays*)

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Root hairs are unicellular extensions present on all root types. They substantially increase root surface area and thereby contribute to water and nutrient uptake. In maize, a small number of *roothairless* mutants have been identified, including *rth1*, *rth2* and *rth3* (Wen & Schnable 1994). In this study we present progress in developing a reference proteome for maize root hairs and an update on the physical mapping of the *rth2* locus.

To define the maize root hair proteome, label-free Gel LC MS/MS was used to identify 2,573 proteins covered by at least two peptides. Blasting against NCBI.nr and other databases, using Mapman Mercator software a functional classification for 96% of these proteins was achieved. Recently, Brechenmacher *et al.* (2009) published a root hair reference proteome of the dicot model soybean (*Glycine max*). Out of 1492 proteins homologs of 898 proteins were present in both datasets enabling comparisons of the root hair proteomes of these species. Compared to wildtype, the *rth2* mutant displays significantly shorter root hairs at all root types 9 days after germination. The *rth2* locus has been mapped to an interval of 280 kb that includes five gene models. After cloning, the *rth2* gene will be subjected to a detailed functional analysis including a comparative analysis of the proteome of mutant versus wildtype root hairs.

1. Wen & Schnable 1994. Analysis of mutants of three genes that influence root hair development in *Zea mays* (Gramineae) suggest that root hairs are dispensable. American Journal of Botany 81: 833-842
2. Brechenmacher *et al.* 2009. Establishment of a protein reference map for soybean root hair cells. Plant Physiology 149: 670-682

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P93

Proteomic dissection of heterosis manifestation in developing maize embryos

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Heterosis describes the superior performance of heterozygous F_1-plants compared to their homozygous parental inbred lines. Heterosis in maize *(Zea mays* L.) is already manifested during early plant development. In this survey, embryos of the inbred lines UH005 and UH250 and their reciprocal crosses (UH005xUH250; UH250xUH005) were phenotypically characterized at different time points (25 and 35 days after pollination) in order to characterize the degree of heterosis in the reciprocal hybrids. Subsequently, the embryos of the four genotypes were subjected to a 2D-PAGE proteome analysis in order to identify proteins that are non-additively accumulated in hybrids. Mass spectrometric identification and characterization of 141 proteins that were non-additively expressed in at least one of the reciprocal hybrids is under way. Initial results will be presented.

P94

Regulation and role of the CDK/RBR/E2F pathway in maize transformation and endosperm development

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We have investigated how the cyclin-dependent kinase (CDK)/ retinoblastoma-related (RBR)/E2F pathway is regulated during maize cell transformation and endosperm development, and how it influences these processes. RBR proteins are conserved cell cycle gatekeepers that function through the inhibition of E2F transcription factors. We discovered two types of maize RBR genes, RBR1 and RBR3, which differ in terms of structure, regulation and function. Phylogenetic analyses indicate that these genes may be distinctive features of the Poaceae. Inhibition of RBR1 activity in embryogenic callus stimulated E2F-dependent gene expression, cell proliferation, and transformation. However, it also resulted in increased RBR3 RNA, indicating that RBR1 represses RBR3 expression. The relationship between RBR1 and RBR3 raised the question of whether these two genes have redundant functions. We found that RBR3 plays a positive rather than a negative role in DNA replication, cell transformation, and the expression of the MCM family of DNA replication factors. These features are a paradigm shift in RBR gene function and appear to be unique within the RBR gene family. They suggest the existence in maize and related cereal crops of specific RBR/E2F-dependent pathways impinging on the cell cycle and development. In the endosperm, RBR1 limits E2F-dependent gene expression and cell cycle activity during the endoreduplication phase of development. Down-regulation of RBR1 by RNAi resulted in an increased endoreduplication phenotype. RBR proteins may be inactivated through CDK-mediated phosphorylation, but the identity of the responsible kinase in maize is unclear. CDKA;1 function is important for endoreduplication in the endosperm, and we obtained genetic evidence suggesting that CDKA;1 exerts its function by inhibiting RBR1. During endosperm development, different CDKs and their cyclin partners display specific patterns of expression, kinase activity, and stability. Our results indicate that the endoreduplication phase of endosperm development is characterized by cyclin stabilization due to inhibition of proteasome-dependent proteolysis.

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P95

Regulation of the HD-ZIP IV transcription factor ZmOCL1 by a small RNA

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ZmOCL1 is the founding member of the maize *ZmOCL* (*Zea Mays Outer Cell Layer*) family encoding plant-specific HD-ZIP IV transcription factors. It is expressed in the epidermal layer of the embryo and organ primordia, suggesting a role in the differentiation and maintenance of epidermal cell fate. The existence of a conserved 21 bp sequence in the 3'UTR of the majority of the *HD-ZIP IV* genes from maize, rice and Arabidopsis suggested a potential regulation of these genes (including *ZmOCL1*) by a small RNA. This would be a novel one since no small RNAs matching the 21 bp target sequence were found in existing databases. First experimental evidence for the existence of such a small RNA in maize was obtained by Northern blot. Hybridization of low molecular weight RNA with a radio-labelled probe complementary to the putative small RNA regulating *ZmOCL1* revealed a band in the 22-24 nucleotide size range in several maize tissues such as tassel and ear. We called this small RNA *miR1*. RLM-5'RACE experiments did not allow us to detect any *ZmOCL1* mRNA cleavage products within the putative *miR1* binding site, suggesting that *miR1* does not regulate its target genes by transcript cleavage. On the contrary, a preliminary analysis of *miR1* activity in planta using a GFP sensor system, in which the 3' end of the GFP coding sequence was transcriptionally fused to the entire 3'UTR of *ZmOCL1* (including the *miR1* target sequence) suggests that *miR1* could regulate its target(s) through translational repression.

P96

Revisiting the hetero-fertilization phenomenon in maize using molecular markers

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Hetero-fertilization (HF) is an abnormal fertilization process that occurs when the egg cell (n) and the central cell (or polar nuclei - 2n) of the same ovule are fertilized by genetically different sperm cells released from different pollen grains. Improving our understanding of HF has been constrained by the low number of morphological markers for detection of HF. However, the recent development of a seed DNA-based genotyping system (Gao et al. Mol Breed 22:477-494, 2008) has facilitated routine large-scale identification of HF events by comparing the genotypic difference between endosperm and embryo from any cross. In this study, a diverse set of segregating material was used including seven F2 populations and progeny from four three-way crosses. Moderate to large population sizes (each with 353 to 1024 individuals) were used for analysis of endosperm and embryo (leaf) DNA samples. Simple sequence repeat (SSR) marker analysis indicated an HF frequency of 0.28% to 4.36%, with an average of 2.32%. Analysis of three-way crosses enabled HF events contributed by male and female gametes to be distinguished for the first time. A higher proportion of HF events across both types of population was contributed by the pollen gametes, while the HF contributed by maternal gametes was relatively low (0.28%-1.30%). Four HF plants identified by one SSR marker from each of two F2 populations were confirmed by genotyping with a 1536 SNP chip. The results also confirmed that use of one polymorphic marker can only reveal 50% of the HF events and hence the real HF ratio can be inferred by doubling the HF ratio estimated by one marker. From these results it is concluded that the effect of HF on the accuracy of marker-assisted selection using seed DNA-based genotyping is negligible.

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P97

Role of *Lxm1* in leaf patterning and development

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Lxm1-O is a dominant EMS induced mutation in maize that causes pleiotropic developmental phenotypes that vary depending on inbred background. The most obvious defect is the presence of a flexible midrib. In addition, in *Lxm* mutants the blade/sheath boundary is displaced distally into the leaf blade, a phenotype reminiscent of that caused by ectopic knox expression. In W22 inbred, *Lxm* causes the partial adaxialization of leaves as evidenced by sectors of adaxial tissue in lower leaf surface surrounded by regions of ectopic blade outgrowth. These observations indicate that *Lxm1-O* affects mechanisms involved in leaf determination and patterning. We further characterized the role of *Lxm* in these processes by analyzing its interaction with *rs2*, *Rld1-O* and *lbl1*. Like *Lxm1-O*, mutation of the myb-domain protein RS2 leads to ectopic expression of knox genes in developing primordia resulting in the formation of leaves with a characteristic rough sheath phenotype. In *Lxm1-O rs2* double mutants this sheath phenotype is nearly completely suppressed, whereas the *Lxm1* blade phenotype is slightly enhanced. In maize, abaxial/adaxial patterning requires the activity of *Rld1* and *lbl1*, which encode a class III HD-ZIP transcription factor and a homolog of SGS3, respectively. *Rld1-O* single mutant shows adaxialized leaves due to ectopic expression of *Rld1* caused by insensitivity to miR166 downregulation. *Lxm1-O* and *Rld1-O* enhance each other, resulting in a severe adaxialization of the leaf. Partial abaxialization of the leaf as in *lbl1-ref* is frequently associated with bifurcation of the blade along the midrib. This mild polarity defect is suppressed in *Lxm1-O lbl1* plants. Efforts to clone *Lxm1* by a map-based approach are ongoing. *Lxm1* maps to a region spanning 100 kb on chromosome 3, and the candidate genes in the interval are being analyzed.

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P98

Some thoughts on the efficiency of doubled haploid maize production *in vivo*

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In traditional maize breeding the development of new homozygous cultivars is a very time consuming process and can take up to seven generations of selfing. Therefore, the production of doubled haploids (DH) represents an attractive alternative with respect to saving time and obtaining a true-breeding line. In spite of its application in breeding programs the efficiency of producing doubled haploids has to be improved. This goal may be achieved by optimizing two steps: 1. The frequency of haploid induction, 2. The efficiency in doubling of chromosome sets of haploids to obtain DH plants.

In maize, haploid plants are produced via pollination of diploid plants with pollen of an inducer line. However, haploids only occur at low frequencies. Furthermore, the mechanism of haploid induction is poorly understood. Therefore, it might be helpful to study this process. A better understanding of the mechanism could lead to the improvement of this step. Towards this goal, currently experiments are conducted with isolated embryo sacs. Moreover, increasing the efficiency of the chromosome doubling rate could further improve the output of doubled haploids. Possible pretreatments could be the synchronization of cell division and/or the enhancement of the penetration of blocking agents into maize tissue. Furthermore, early-testing of putative doubled haploid plants could assist in screening. For early testing, we have demonstrated that a shift in the ploidy level may be detected in shoot apical meristems of developing plants two days after application of a mitotic block. Finally, alternatives to colchicine to block mitosis could be developed. Using early testing of developing seedlings it was shown that colchicine could be substituted by herbicides like APM, oryzalin, or pronamide. Further research will be directed at improving the efficiency of double haploid production in maize.

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P99

Source leaves of the maize *indeterminate1* flowering time mutant have elevated carbohydrate levels without a concomitant increase in photosynthetic rate.

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The INDETERMINATE1 (ID1) transcription factor is a key regulator of the transition to flowering in maize. Loss of function *id1* mutants make many more leaves than normal plants and flower very late. *ID1* is expressed exclusively in immature leaves, where it is believed to control the production or transmission of leaf-derived florigenic signals. Previous microarray analysis showed that a significant proportion of genes up-regulated in *id1* mutants compared to normal plants are associated with carbohydrate metabolism, including photosynthesis (Coneva *et al*., 2007). As a follow-up to this finding, and in light of earlier physiological studies that suggest a possible connection between carbohydrate flux and the floral transition in higher plants, we are investigating several aspects of the physiology and metabolic status of the *id1* mutant. Mature source leaves of mutant and normal-flowering plants at key developmental stages were analyzed for their capacity for gas exchange, electron transport, and the diel content of major carbohydrates. Our results to date show irradiance-dependent differences in leaf carbohydrate content between *id1* and normal plants in the absence of a significant variation in photosynthetic rate. Current experiments are aimed at elucidating the potential link between the late-flowering phenotype of *id1* and carbohydrate metabolism, including carbon export and utilization.

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P100

Strategies for the isolation of the *Shootmeristemless* (*sml*) gene in maize

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The *Shootmeristemless* (*sml*) and *distorted growth* (*dgr*) genes are involved in maize organogenesis. A mutation of the *sml* gene disrupts shoot apical meristem maintenance and lateral organ formation. Introgression of this mutation in different genetic backgrounds has highlighted the epistatic interaction between *sml* and the unlinked *dgr* gene. Seeds homozygous for both *sml* and *dgr* have a shootless phenotype whereas *Dgr/-sml/sml* seeds produce plants with developmental abnormalities (dab phenotype). The morphological and histological analysis of the dab phenotype reveals a variety of plant abnormalities affecting different plant organs, including leaf, inflorescence, root and stem; the severity of the defects may vary widely within a single mutant plant. Mutations in the *leafbladeless1* (*lbl1*) gene, that is involved in the biogenesis of *trans*-acting small interfering RNAs, lead to a very similar phenotype. However the two mutations define two different genes. It is conceivable that *sml* and *lbl1* are located in the same pathway. This hypotesis is reinforced by the observation that the *sml* gene lies in a syntenic region with *SHOOT ORGANIZATION1* (*SHO1*) in rice. *SHO1* encodes for a DCL4 protein, which is responsible for the processing of 21-nucleotide *trans* acting –siRNA. The sequence of the orthologue of *SHO1*, *ZmDCL4* has been reconstructed. Its 5353 bp long transcript, is ubiquitously expressed and semi-quantitative RT-PCR analysis reveals a decrease of the *ZmDCL4* transcript level in the mutant apex. Evidence for the correspondence between *sml* and the *ZmDCL4* locus were provided by allelisms test between *sml* and other mutants carrying a lesion in this gene. Our results reveal that the *ta*-siRNA pathway is affected in the dab mutant: the target gene *arf3a* is upregulated in the mutant apex and this change is correlated with a decreased level of *arf3a* cleavage products.

P101

Study of maize male floral anthesis by cryo-SEM

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Anthesis of maize male flower, as in the majority of Poaceae, consists of a series of rapid developmental events; these include lodicule expansion, glume and lemma outward bending (floret opening), filaments elongation, anther pore opening, ballooning of microsporangia, and pollen shedding. The well orchestrated physiological control results in preferential water movement and action of surface tension, which then cause rapid movement. Cryo SEM, superior in identifying air space and liquid space within tissue, was used to study the rapid-frozen spikelets of Ohio 43 and Gaspe Flint. Our observations revealed that, at the onset of anthesis, the distal end of lodicule has a tangential fold, which allows significantly radial but minimum longitudinal expansion during anthesis, consequently, glumes and lemma are pushed outward and florets are open; anther epidermal cells are highly turgid; each microsporangial locule is enveloped by a layer of each of the tapetal film, endothecium and epidermis; and cells of intermicrosporangial stripe 1 (IMS1) are loosely connected with the underlying connective cells which have large intercellular space. During anthesis, filament elongation proceeded steadily due to cell elongation, but epidermal cells at the two ends remain small. When the filaments are well extended, the epidermis of anther apex, which lacks of connective, starts to shrink, which results in the anther pore opening; however, anther dehiscence is not yet completed until the paired microsporangial locules become confluent (ballooning). Force generated by the epidermal cells, while it begins to lose turgor, acts on the underlying endothecium layer and results in the separation of IMS1 from underlying connective, the site with the physical weakest point. As the IMS1 separating from the connective, tapetal film is torn and ballooning takes place. During ballooning, the tightly packed pollen grains are released due to the increase of space and thus are possible for dispersal. Except at the portion where the pore is formed, IMS1 remains intact throughout the dehiscent process.

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P102

The *empty pericarp4 gene* is required in post-germinative maize development

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The *empty pericarp4* (*emp4*) gene encodes a mitochondrion-targeted pentatricopeptide repeat (PPR) protein that is necessary for the correct regulation of mitochondrial gene expression in the endosperm. To gain inside into the role exerted by EMP4 during plant development we have adopted two strategies.

Homozygous mutant *emp4* embryos are retarded in their development and unable to germinate; therefore homozygous mutant seedlings were obtained from the cultivation of excised immature embryos on a synthetic medium. After 20 days of culture, 40% of the mutant embryos germinated and reached the coleoptilar stage; among them only 10% produced seedlings that developed the first leaf and few seedlings reached the second leaf stage.

Moreover a chromosome breaking stock with the *Ac* transposon and a double *Ds* element located on the long arm of chromosome one, where *emp4* resides, had been crossed with *+/emp4* plants. Chromosome breakages, induced by Ds in somatic tissues, are expected to produce chimeric plants bearing, in a wild-type background, sectors hemizygous for the *emp4* mutation. Clearly distinguishable yellow sectors were isolated from wild-type green leaves and their hemizygous genotype confirmed by PCR.

Changes in the subcellular structure were highlighted from the comparison of wild-type and mutant tissues by means of transmission electron microscopy. In *emp4* mutant leaves, mitochondria as well as chloroplast populations were significantly reduced and both organelles displayed a less organized structure. Tissues derived from embryo rescue have also been studied through semiquantitative RT-PCR analysis to compare the expression profiles of mitochondrial genes that are the putative EMP4 targets.

In summary, these data suggest that *emp4* gene action is required for the correct development of post-germinative leaf tissues.

P103

The majority of maize caryopsis cytokinins accumulate in the pedicel

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Cytokinins are a major group of plant hormones, associated with various plant developmental processes. Since the discovery of the first natural cytokinin, zeatin, in maize caryopsis, cytokinins have been thought to be involved in early caryopsis development. Roughly concurrent peaks of mitotic activity in developing endosperm and cytokinin accumulation in the lower part of caryopsis have previously prompted suggestions that caryopsis cytokinins are primarily involved in maintaining mitotic activity in endosperm.

However, our studies indicated that cytokinin peak appeared well before the phase of intense cell divisions in the endosperm. Moreover, we examined the abundance of different cytokinin metabolites and, for the first time, histochemically immunolocalized cytokinins in various parts of developing caryopsis, including pedicel, endosperm and embryo. Immunolocalization data revealed that most caryopsis cytokinins were present in the pedicel region, specifically the placento-chalazal (P-C) layer and the vasculature. The P-C layer is believed to be critical in the post-vascular transport of water, sugars and nutrients to the developing seed, and undergoes fertilization-dependent programmed cell death. Transcript localization of the cytokinin biosynthetic gene isopentenyl transferase 1 (*ZmIPT1*) to the pedicel vascular tissue further corroborated that de novo biosynthesis may contribute at least partly towards cytokinin pool. Comparison of cytokinin content between unfertilized ovules and developing caryopsis in the present work suggested that cytokinin accumulation in the P-C layer was also triggered by fertilization. The possible role of pedicel cytokinins in development of caryopsis will be discussed.

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P104

The *BARREN STALK FASTIGIATE1* AT-hook transcription factor is required for ear formation

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Maize ears develop from axillary meristems formed in the axils of leaves soon after germination. Genes regulating the formation of these meristems have been identified thanks to studies on a class of mutants affected in ear and tassel development, the *barren* mutants. We recently characterized one of these mutants, called *barren stalk fastigiate1* (*baf1*), first discovered in the 1950's, that is impaired in the formation of ears. *BAF1* encodes a transcription factor containing an AT-hook DNA binding domain. Co-orthologs of *BAF1* are found in syntenic regions of Brachypodium, rice and sorghum suggesting that the gene is likely present in all cereal species. Another transcription factor involved in this pathway is the basic helix-loop-helix BARREN STALK1 (BA1). We will present genetic and molecular data on the interaction between *BAF1* and *BA1*, and our ongoing efforts to identify additional genes required for the initiation of maize ear primordia.

Funding acknowledgement: National Science Foundation (NSF)

P105

The *PIN-FORMED* family of auxin efflux carriers in maize

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Polar auxin transport is a unique process mediating a wide variety of plant developmental processes, including organogenesis, tissues differentiation, root initiation, apical dominance, and tropisms. Specific trans-membrane carriers (AUX1/LAX, PGP and PIN protein families) mediate the cell to cell auxin transport, creating auxin gradients that, in turn, control gene expression. Eight PIN proteins (AtPIN1-AtPIN8) were found in Arabidopsis and have been shown to play a rate-limiting role in the catalysis of auxin efflux from cells determining the direction of cell-to-cell auxin flow and, as a consequence, creating the auxin gradients that regulate plant development.

PIN genes are present in genomes throughout the plant kingdom, from the model moss *Physcomitrella patens* to all vascular plants. Phylogenetic analysis of PIN sequences from *Oryza sativa* and *Triticum aestivum* revealed that the monocot *PIN* family is wider and divergent than dicots one, with three or four genes homologous to one Arabidopsis *PIN* gene and with the presence of monocot-specific PIN proteins.

Blast searches allowed the identification of a at least ten members of the maize *PIN* family and preliminary phylogenetic analysis using the newly detected ZmPIN proteins plus rice and Arabidopsis PINs confirmed the widening of monocots *PIN* family compared to dicots one. We indeed identified at least three orthologs of *AtPIN1*, called *ZmPIN1a*, *ZmPIN1b* and *ZmPIN1c*, two genes closely related to *AtPIN2* (*ZmPIN2a* and *ZmPIN2b*) and three putative orthologs of *PIN5* (*ZmPIN5a*, *ZmPIN5b* and *ZmPIN5c*). As previously demonstrate in rice, we identified also in maize two monocot-specific proteins. Semiquantitative RT-PCR expression analysis revealed that newly identified *ZmPIN* genes are differentially expressed during maize embryonic, vegetative and reproductive development. To better understand the role of these genes in controlling seed and plant development we are determining their expression patterns at cell and tissue level.

P106

The Effect of Auxin Combinations on Callusogenesis of Corn Inbred Lines

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Callusogenesis in immature embryo culture is an important experimental system for biotechnological improvement of corn varieties through plant regeneration, somaclonal variation, cell and protoplast culture establishment, and genetic transformation. The production of morphogenic calli can be improved by the optimization of the phytohormone content of the nutrient medium. In this presentation, we describe the nature of callusogenesis in corn inbred lines of different commercial germplasms under the effect of different auxins and their combinations. N6 modified nutrient medium with 1 mg/l 2,4-D was taken as a control for callus initiation. Investigated genotypes were differentiated under the frequency of the formation of morphogenic calli on control medium to genotypes with low responsibility: ДК633 (germplasm Mo17) – 2,1%; ДК366 (germplasm Oh43) – 7,6%; ДК675 (germplasm Lancaster) – 27,4% and genotypes with high responsibility: Chi31 (germplasm Chi31) – 77,6%, PLS61 (germplasm PLS61) – 87,6%, ДК443 (germplasm Iodent) – 93,3%. We investigated the effect of changing 1 mg/l 2,4-D in initiation medium to one of the following auxin substances and their combinations: 1 mg/l dicamba; 2 mg/l dicamba; 0,5 mg/l 2,4-D+0,5mg/l dicamba; 0,5 mg/l 2,4-D+1mg/l dicamba. Stimulating effect of modified auxin content was recovered only for inbred lines with low level of the formation of morphogenic calli. The increase of morphogenic callus production occurred predominantly due to the stimulation of friable callus (type II) formation. The best for morphogenic callus initiation was 1mg/l dicamba instead of 1 mg/l 2,4-D, resulting for ДК633 in 4,2%; for ДК366 in 21,9%; for ДК675 in 54,7% of morphogenic calli. Callus tissues after initiation on 1 mg/l dicamba medium quickly turned to differentiation and regeneration and could not be maintained for a long time. Combination of 0,5 mg/l 2,4-D+1mg/l dicamba was also better than control for morphogenic callus initiation (ДК633 – 9,5%; ДК366 – 11,4%; ДК – 41,0%), but at the same time this combination was very useful for long-term maintenance of morphogenic callus tissues of given genotypes for further application to biotechnological programs.

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P107

The auxin efflux inhibitor NPA causes aberrant embryo and endosperm development, *PIN1* miss-expression and altered auxin accumulation patterns in maize

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Auxin represents a key factor in regulating a multitude of processes during plant growth and development, starting from embryogenesis up to lateral organ formation, meristems maintenance, vascular tissue differentiation and tropisms. All these developmental processes are indeed correlated with the creation of differential auxin distribution in the corresponding tissues or cells. These auxin gradients mainly depend on its polar transport, mediated by specific trans-membrane auxin influx (AUX and LAX proteins) and efflux carriers (PIN proteins).

The combined action of PIN1, PIN4 and PIN7 auxin efflux carriers mediate the creation of differential auxin distributions fundamental for proper Arabidopsis embryogenesis and perturbations of the protein activity (in *pin* loss of function mutants or by chemical inhibition) result in many embryonic defects such as the failure in the establishment of the apical basal polarity or abnormal cotyledon differentiation.

Our results on ZmPIN1 protein localization and auxin accumulation patterns during maize kernel development demonstrated the primary role of PIN1-mediated auxin transport and accumulation for proper maize embryo and endosperm development.

In the absence of specific maize *pin* mutants, applications of the auxin efflux inhibitor N-1-naphthylphthalamic acid (NPA) were used to confirm our results and to study the effects of auxin transport block on *PIN1* expression, auxin accumulation and embryo and endosperm development. All these aspects are seriously affected by NPA treatments that result in the formation of abnormal embryos with miss-specified apical structures. Scutellum morphology and symmetry and vasculature differentiation are also strongly affected by the treatments, while in the endosperm the main developmental defects concern the aleurone layer. All these alterations are accompanied by ectopic *ZmPIN1* gene expression and atypical protein polarization and by the disruption of the auxin gradients inside the embryo and the endosperm.

P108

The pod corn phenotype is caused by the ectopic expression of an STMADS11-like MADS-box gene in the ear of maize

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Tunicate (Tu) is a dominant, gain-of-function mutation of maize that causes the famous pod corn phenotype. The predominant feature of pod corn is a foliaceous elongation of the glumes, so that they cover the kernels in the ears. Pod corn has also a fascinating cultural history reaching back into precolumbian times. Some classical papers of 20th century maize genetics reported that the mutant Tu locus is complex, but molecular details remained unknown. We have molecularly cloned the Tu gene and show that pod corn is caused by a cis-regulatory mutation and duplication of ZMM19, an STMADS11-like MADS-box gene encoding a putative transcription factor. While the wild-type locus contains a single copy gene that is only expressed in vegetative organs, mutation and duplication of ZMM19 in Tu leads to ectopic expression of the gene in the inflorescences, thus conferring vegetative traits to reproductive organs.

P109

The role of auxins in the development of defective endosperm-18 (de18) mutant mutant of maize: regulation of cell division and endoreduplication

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The maize mutant defective endosperm-18 (de18) contains 15 times lower auxin levels, accumulates 35 percent less dry matter compared to the wild type. The plant hormones auxins and cytokinins are involved in regulation of mitosis and endoreduplication, cellular key processes during seed development. To investigate whether the reduced endosperm of de18 is due to impaired cell division and endoreduplication process, as a consequence of the low auxin levels, wild-type B37 and de18 kernels were analyzed at 8, 12 and 16 DAP. Nuclear endoreduplication level, number and size of cells have been measured with the optical miscroscope and computer image analysis using the 3D model developed for maize endosperm. Total number of cells of different endoploidy levels in the endosperm was estimated using the 3D model developed for maize endosperm (Vilhar et al., 2002 Plant Physiol 129:23-30).

At 8 DAP the endosperms of both genotypes contained a similar number of cells, most of them belonging to the 3C and 6C ploidy classes. In endosperms of later stages (12 and 16 DAP) we observed progressively more endopolyploid cells (12C and higher) that were located in the central part of the endosperm, whereas the outer layers contained small cells with low nuclear DNA contents. The maximum observed endoreduplication level of cells was 192C in both genotypes. At 12 and 16 DAP the de18 endosperm contained a similar number of 3C and 6C cells as compared to wild type, but the number of endopolyploid cells (12C to 192C) was much lower. The volume of de18 endosperm was lower at 12 and 16 DAP as a consequence of a lower number of highly endoplyploid cells. The most significant differences between de18 and B37 were detected at 12 DAP, where the mutant showed a deficiency in the ploidy level, number and volume of cells. These results suggest that the mutant is characterized by a defective cellular proliferation and progression of endoreduplication, associated to reduced cell volumes, contributing to a decreased endosperm development.

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P110

Three *tasselless* (*tls*) loci are essential for vegetative and reproductive development in maize

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Plant development involves highly regulated processes of growth and differentiation. Both cell division and cell expansion are critical for normal vegetative and reproductive development, and disrupting either of these processes can result in severely altered growth. Phenotypic analysis of the maize *tasselless* (*tls*) mutants has revealed potential roles of the tls genes in regulating these aspects of development.

tasselless1 (*tls1*) mutants show defects in both vegetative and reproductive growth which vary in severity depending on the allele and the environment. *tls1* plants are shorter in height than normal siblings and mutant leaves show distinct developmental defects. During reproductive growth, both tassel and ear inflorescences of *tls1* mutants are significantly reduced or absent. A characteristic of *tls1* mutants is the presence of a short ball-shaped ear within the husk leaves, indicative of defects in apical meristem maintenance.

tls1 maps to bin 1.07 and has been cloned using a positional cloning approach. Based on phenotypic analysis and gene identity, we hypothesize that *tls1* plays a key role in cell division and/or cell expansion throughout maize development. Additional *tls*-like loci have been mapped to bins 2.02 and 3.09. Cloning and analyses of these genes will further our understanding of how the *tls* loci regulate critical aspects of maize development.

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P111

Toward an understanding of the function of DISCOLORED1 (DSC1) in maize kernel development

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ADP-ribosylation GTPase-activating proteins (ARF-GAPs) function in endomembrane trafficking and belong to a large family of proteins that are conserved in many species including humans, *Drosophila*, yeast, *Arabidopsis*, and maize. *discolored1* (*dsc1*) putatively encodes one of the thirty-six predicted ARF-GAPs found in maize. The *dsc1* mutation, which was identified in a screen of *defective kernel* mutations arising from a *Mutator* stock collection, segregates brown malformed kernels. Phenotypic analysis of dsc1 mutant kernels harvested from 8 days after pollination (DAP) through 16 DAP indicates that despite a delay in the development of both the embryo and the endosperm no morphological defects can be observed in either tissue type. However, the onset of severe deformities and deterioration in both tissues occurs by 20 DAP. This onset of dsc1 mutant embryo degeneration correlates with the pattern of *knotted1* (*kn1*) expression, which is equivalent to that of wild type siblings until 16 DAP but is absent from mutant embryos thereafter. The full length *dsc1* gene aligns to BAC AC197554 and is predicted to encode an 823 amino acid ACAP type ARF-GAP. The putative DSC1 orthologue in *Arabidopsis* is VASCULAR NETWORK3/SCARFACE (VAN3/SFC), which functions in the recycling of the auxin efflux protein PIN1 and is necessary for polar auxin transport. Currently we are performing complementation tests in *Arabidopsis* van3/sfc mutants, over-expressing truncated DSC1 in *E. coli* to perform an ARF-GAP activity assay, and using YFP tagged transgenic lines to determine if DSC1 functions in the recycling of endomembrane trafficked proteins such as PIN1. Our future prospects include generating additional mutant alleles through reverse genetics and identifying the sub-cellular localization of DSC1 through transient expression of GFP tagged DSC1 in *N. benthamiana*.

P112

Transcriptome and proteome support for organ-specificity of *Ustilago maydis* tumor induction in maize

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The biotrophic fungal pathogen *Ustilago maydis* elicits tumor formation by redirecting development of immature vegetative and floral primordia into a tumor pathway. Injections of wild-type (FB1 + FB2 or the solopathogenic SG200 strain) *U. maydis* into the ~2 cm tassel inflorescence through the leaf whorl of 5-week old plants demonstrated that tumors can form on maturing leaves and all floral organs. A sharp demarcation was commonly observed between tumor-forming regions and areas with normal spikelet maturation and pollen shed; this suggests that both *U. maydis* signals and host responses are restricted spatially. Interestingly, male sterile mutants with defects in anther cell wall differentiation (i.e., prior to meiosis) were unable to form anther tumors. The growth potential mutant *Dwarf8* as well as *spi1* and *fea2* failed to produce tassel tumors while the *Knotted1* mutant produced copious, abnormally large leaf and tassel tumors. On the basis of these phenotypic differences we hypothesized that *U. maydis* tumor formation requires organ-specific gene expression by both partners. A two-organism transcriptome profiling platform was used to identify stage-, organ-, and organism-specific gene expression. A majority of the gene expression differences detected showed that both maize and *U. maydis* express genes in an organ-specific pattern. These data provide strong support for our hypothesis and may explain the organ-specific nature of many plant pathogen infections. Whole proteome experiments are being conducted on infected versus mock tissue samples. Mass spectrometry profiles have already identified over 2000 maize proteins per sample, several hundred of which are detected only in infected or mock tissues. This work was supported in part by an SGER grant from the NSF and the Savitsky Endowment.

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P113

Transcriptome sequencing and expression profiling during maize inflorescence development

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In both maize ears and tassels, branching patterns are determined by developmental fate in a series of axillary meristems, which initiate progressively along the inflorescence. In this work, we analyzed genome-wide expression profiles during three sequential stages of maize flower development in ears using an RNA-seq approach. Individual libraries were constructed from developing spikelet samples enriched for spikelet pair, spikelet, or floral meristematic tissue. From each sequenced library, approximately 6M good quality, paired-end RNA-seq reads mapped to the assembled maize reference genome using a set of high-confidence, filtered transcript models. A total of 39,681 unique transcripts were identified among all libraries and their normalized expression values (Reads Per Kilobase of exon per Million) spanned 5 orders of magnitude with 37% having values higher than 5 RPKM. We used the quantitative RNA-seq data to identify groups of co-expressed genes during early flower development and distinguished between specific transcript variants that were differentially expressed in these tissues. Gene trees available from Ensembl Compara were used to leverage information from orthologs in Arabidopsis and rice in the functional annotation of maize genes. Based on these analyses, we identified candidate transcription factors as potential regulators of flower development as well as components of entire biochemical pathways. We also compared the expression data from young ear libraries to those of developing tassels and to parallel microarray experiments. The transcript-specific profiles generated here, along with the current integration of parallel datasets from loss-of-function mutants in meristem determinacy, provide a foundation for functional analysis of gene networks underlying inflorescence architecture, an important agronomic trait. A.L.E. is funded by award #0805655 NSF Postdoctoral Fellowship in Biological Informatics. D.J., A.G. by the NSF Plant Genome Program.

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P114

Transgenic Research in Maize using Gateway Technology

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Sequencing of the maize genome is a major achievement, however transgene technology and efficient transformation are equally important for the species to be fully exploited as a model for cereals. So far, functional analysis in maize merely depended on gene knock-outs, that could be retrieved from large collections of insertion lines generated with endogeneous transposon. However, in the post-genomic era of maize, genes of interest can be identified, by sequence information, for functional analysis through transgenic silencing approaches. In addition, translational research in maize of genes with putative beneficial effects will be greatly facilitated by transgenic research.

We developed an efficient and standardized maize transformation method based on the following criteria: (1) Agrobacterium tumefaciens co-cultivation was preferred over particle bombardment because usually intact T-DNA's are transferred at low copy number, (2) a maize inbred line was favoured over a hybrid line because homogeneous progenies allow transgene testing already in T1 or T2, (3) Gateway vectors were optimized for use in monocots and provide a toolbox for rapid gene cloning. Co-cultivation of inbred immature embryos and phosphinotricin selection conditions were optimized to yield transformed T0 shoots at efficiencies up to 15 %. The presence and activity of overexpression and silencing T-DNA constructs were verified at the molecular level in T0 and T1. T1 progenies were obtained 7 months after co-cultivation and showed a Mendelian segregation of single T-DNA loci predominantly, indicating stable transformation and clonal origin through somatic embryogenesis.

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P115

Transgenic plants producing non-transgenic pollen – A new confinement method for maize?

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One of the major setbacks and concerns against transgenic plants is the uncontrolled proliferation of transgenic sequences into the environment. One of the main reasons is the dispersion of transgenic pollen, pollinating non-transgenic plants or even cross-breeding into wild species.

The solution for this plight is a specific removal of transgenic sequences during the pollen-development. Because of the need for breeding and seed production, a dormant construct is transformed. The construct consists of an active gene of interest region, coding the desired transgenic properties. Additionally the construct carries an inactive excision region, containing two different recombinase genes and their regulating promoters.

The spacer inactivating the excision region consists of a heat shock promoter, regulating a recombinase bracketed by its specific recognition sites. After a heat shock, the recombinase will excise itself and the corresponding promoter. This combines a pollen specific promoter with a second recombinase. During the pollen development, the expression of the second recombinase will lead to the removal of whole construct, leaving only the border sequences from the transformation and a single recombinase recognition site.
Neither coding nor regulating regions will be left in the pollen, which will lead to a significant reduction of unchecked dispersion of transgenic material into nature.

Here we represent first data from maize transformation and excision experiments.

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P116

You'll never walk alone: ZmMRP-1 is not the only Myb related transfer cell specific transcription factor

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In previous works we have characterized the role of ZmMRP-1 in the development and differentiation of the transfer cell (TC) layer of the maize endosperm. ZmMRP-1 is a TC-specific transcription factor that contains a single MYB-related DNA binding domain. Within the TC the protein transactivates a number of TC-specific promoters through the interaction with a specific DNA motif that we have called TC box. A number of Basal Endosperm Transfer Cell Layer (BETL) specific genes controlled in this way by ZmMRP-1 have been characterized. Although the ectopic expression of the gene is lethal in maize and other species, we have recently shown that its expression in the aleurone promotes the transformation, albeit transient and partial, of the MRP-1 expressing cells into TC.

Several evidences suggested that ZmMRP-1 might be just a piece of a more complex regulatory network operating at the TC layer: (1) The transformation of the aleurone cells expressing the transcription factor was neither complete nor permanent. (2) The promoter of ZmMRP-1 is TC specific in maize but labels additional transport-related areas in Arabidopsis and tobacco. (3) Transcriptomic studies indicate that not all the members of some BETL families are regulated by ZmMRP-1.

We have explored the recently completed maize genome sequence in search for possible ZmMRP-1 paralogs, finding 5 highly related genes distributed throughout several maize chromosomes. An additional gene, not yet annotated in the maize genome, was found in a TC-specific cDNA library. We will show here a detailed analysis of the expression pattern of the 7 genes in the maize plant and discuss its implications in connection with the previously known data on the expression of ZmMRP-1 in heterologous species.

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P117

ZCN8 is likely a central integrator of flowering time signals in maize leaves

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Flowering is arguably one of the most important developmental decisions made by maize, or any other cereal, as the floral structures are the ultimate source of agronomic products. Plant vegetative and floral structures both arise from the shoot apical meristem (SAM). The developmental trajectory of the SAM is governed by endogenous cues that originate in leaves and by environmental signals detected in leaves, instead of by signals from within the meristem itself. The integrated response from leaves is communicated to the SAM by means of a mobile florigen protein, which in Arabidopsis is embodied by FLOWERING LOCUS T (FT). An important role of the meristem-extrinsic florigen signal is to coordinate floral development with environmental cues such as photoperiod and temperature. As the primary messengers of floral developmental signals from the leaf to the SAM, FT-like genes are the focal point and site of integration for all the upstream signaling pathways that ultimately determine flowering time. Consequently, a powerful means to find the components of the signaling network in maize that controls flowering time is to identify genetic loci that modify expression of FT-like genes. Therefore, we have focused on identifying a functional FT-like ortholog in maize. Our conclusion is the Zea mays *CENTRORADIALIS* (*ZCN*) *8* gene is likely to encode a protein with an FT-like activity. The temporal, spatial, developmental, and photoperiod-dependent patterns of *ZCN8* expression are consistent with a florigen gene. Furthermore, the rise of *maize MADS-box4* (*ZMM4*) transcript in shoot apices, which marks the onset of floral transition at the SAM, is immediately preceded by a comparable increase of *ZCN8* expression in leaves. The correlation between *ZCN8* and *ZMM4* expression is evident in genetically diverse inbreds that range from early to late flowering. In addition, *ZCN8* transcript levels in young plants accurately predict the eventual flowering time of these plants; thus, *ZCN8* expression is a molecular readout of maize flowering time. As such, *ZCN8* represents a powerful phenotyping tool, which we will exploit to identify genetic loci specifically involved in the early signaling events in leaves that govern initiation of flowering in maize.

Funding acknowledgement: United States Department of Agriculture (USDA)

P118

barren stalk*3112 is a new maize mutant essential for tassel and ear development

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Maize reproductive development results in the production of a tassel (which produces the male flowers) and the ear (which produces the female flowers). Normal tassels consist of a main spike with several long branches covered with spikelet pairs. Each pair of spikelets contains four florets, which produce the pollen. The ear shoot develops from the main stalk, in the axil of a leaf, several nodes below the tassel. The ear produces the female florets that, when fertilized, develop into kernels. The barren class of mutants is defective in tassel and/or ear development. Key members of this group include barren inflorescence2 (bif2), which encodes a serine/threonine kinase required for polar auxin transport and barren stalk1 (ba1), which encodes a bHLH transcription factor. ba1 acts downstream of bif2 in reproductive development and bif2 has been shown to interact with, and phosphorylate, ba1 in vitro. Here, we present the characterization and mapping of barren stalk*3112 (ba*3112), a newly identified recessive maize mutant defective in tassel and ear development. In some backgrounds, ba*3112 produces a normal tassel and no ear, or rarely, a barren ear. Upon introgression into the B73 background the phenotype becomes more severe. In B73, ba*3112 tassels produce few branches and spikelets; many of the spikelets are single instead of paired. ba*3112 mutants in B73 never produce an ear or an ear groove. The phenotype of ba*3112 is very similar to ba1; however they are distinct loci, as they map to different chromosomes. ba*3112 phenotypic characterization, double mutant analysis and positional mapping data will be presented.

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P119

compact plant2 (ct2) encodes the α-subunit of a heterotrimeric GTPase in maize and is required for the regulation of meristem size

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Meristems have the remarkable ability to regulate their size during development, by balancing stem cell proliferation with the incorporation of daughter cells into primordia. We are particularly interested in studying this process in maize, where a large number of meristem proliferation, or "fasciated" mutants are available, including the mutation compact plant2 (ct2). ct2 mutants are significantly shorter than their wild type siblings, but more interestingly ct2 mutants are characterized by enlarged vegetative and inflorescence meristems, resulting in severely fasciated ears and in an increased tassel spikelet density. We used a map-based cloning approach to isolate the compact plant2 (ct2) mutation and find it encodes the α-subunit of a heterotrimeric G Protein. Heterotrimeric G proteins are secondary messengers composed of three dissimilar subunits: Gα, Gβ, and Gγ. In Arabidopsis and rice Gα is encoded by a single-copy gene and is involved in multiple aspects of growth and development, including cell proliferation and cell elongation. Initial analysis of transgenic plants expressing CT2 fused to YFP indicates that CT2 protein is strongly expressed within meristems. Transcript-level analysis of the GA synthesis rate-limiting enzyme GA20ox shows that it is significantly down regulated in ct2 mutants, providing a novel link between GA action and meristem size regulation. In addition, our analysis of ct2/fea2 double mutants suggests, that ct2 identifies a pathway for regulation of meristem size partially shared with the fasciated ear2 signaling.

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P120

fasciated ear3: mutant characterization and map based cloning in maize.

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Inflorescence meristem development is critical for patterning and maintaining maize inflorescences. The recessive fasciated 3 (fea3) mutant of maize perturbs both male and female inflorescence development. The mutants develop fasciated ears with extra number of kernel rows, irregular kernel rows, and develop tassels with higher spikelet density and an increase in main rachis diameter. SEM analysis showed that the inflorescence meristem in fea3 was flattened and ring shaped compared to the wild type, compact conical structure. Thus, enlargement of the fea3 ear is likely caused by an increased inflorescence meristem size. To identify the genes involved in fasciation, we are using map based cloning approaches. Using a total of 947 recombinants, we have narrowed down the fea3 region to 5 BACs, between c0267M03 and c0376H14 on contig 117 of chromosome 3. By bulked segregant mapping of a number of other maize fasciated mutants identified from EMS or Mutator screens, we have recently identified two additional putative fea3 alleles. Five additional candidate alleles have also been identified by targeted EMS mutagenesis and screening. Sequence analysis of candidate genes within the 5 BAC interval are being performed to identify the fea3 gene. Further work will include a detailed phenotypic characterization of fea3 mutants, and expression analysis to elucidate the fea3 function and mode of action.

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P121

Identification of *Mutator* Insertion Sites by sequencing the MTM population

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The MTM (maize-targeted mutagenesis) population was generated using high-copy DNA transposons (*Mutator*) which were mobilized and then stabilized genetically by crossing to *Mu-inhibitor* (*MuI*) lines. Due to the high mutation rate of *Mu* transposons, a relatively small population of MTM lines can saturate most genes with new alleles. Here we show that *Mu* insertion sites can be identified via Illumina high-throughput sequencing technology. Pooled genomic DNAs of F1 MTM plants have been processed by a modified GenomeWalker library generation protocol. Paired-end sequencing has been applied to sequence flanking *Mutator* insertion sites. From previous studies (Alleman and Freeling, 1986), we anticipated that there should be at least 50 new germinal insertions in each plant, along with a similar number of parental insertions. We performed a pilot study with 3 pools (1 row and 2 columns) of 48 plants each to identify new germinal insertion sites. A total of 5 million reads were obtained from the 3 pools which could be distinguished by barcodes, and 50-75% of them had both authentic MuTIR and adapter/barcode ends. Among the authentic reads, 50% were mapped to a unique location in B73 maize reference genome. Each grid consists of 48 rows and 48 columns, with each plant represented in the grid as a row-column intersection. The 1 row and 2 columns intersected at 2 individual plants. Bioinformatically, we could find about 25 new germinal insertions per plant and verify candidates by locus-specific PCR reactions. 25-50 new germinal insertion sites per plant correspond to 50,000-100,000 independent insertions in a single grid (96 pools) of 2304 plants. It is estimated that MTM resource has more than 1 million independent new insertions of *Mutator* elements in the 400Mb gene space, or more than one insertion per kb.
Alleman M and Freeling M (1986) Genetics 112(1):107-19

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P122

A trancriptome profile of basal endosperm transfer cell layer (BETL) in W22 developing seeds.

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Developing endosperm in maize is comprised of four well identifiable cell types: aleurone layer, basal endosperm transfer layer (BETL), embryo-surrounding region, and storage cells that contribute > 95% of the total mass of the endosperm. BETL is the first filial cell layer at the base of the endosperm adjacent to the maternal pedicel tissue, and is best developed as transfer cells in maize among all cereal caryopses. Transfer cells are ubiquitous in plant tissues and are uniquely decorated by wall-in-growths (WIGs) that greatly increase plasma membrane area which is believed to increase their capacity for solute acquisition and transport from the mother plant in developing seeds. Our recent ultra-cellular level analyses show that maize BETL is also ideally suited for polarized polysaccharide secretion in developing endosperm (*Plant Physiol.* 2009, 151:1366). Several lines of genetic evidence in maize indicate that a BETL with robust WIGs is critical for a healthy and viable seed development. Although several BETL-specific genes are described in maize, there is no global analysis or a catalog of all genes expressed in this cell type. Here we describe a simple manual cryosection method to micro-dissect BETL from 12 DAP kernels to prepare RNA for cDNA synthesis, 454 sequencing and EST assembly. Validation of the BETL-specificity of some of the 454 sequences by q-PCR and by *in situ* hybridization established that the approach was successful. We identified 2473 ESTs with an average length of 218 bases; their functional annotation and categorization showed that the largest proportion of the BETL genes were engaged in functions related to mitochondrial activity, sugar and amino acid transport and metabolism, stress response proteins, hormone metabolism, protein metabolism, nucleotide and protein binding activities and defense functions (flavonoid and isoflavonoid biosynthesis). Detailed analysis of these results in terms of EST analyses, metabolic pathways and their functional classification will be discussed.

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P123

A Major QTL for Resistance to *Fusarium* Stalk Rot in Maize

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Fusarium graminearum Schwabe is one of the predominant fungal pathogens worldwide that cause stalk rot in maize. With the BC1F1 mapping population prepared from the cross of '1145' (donor parent, complete resistant) and 'Y331' (recurrent parent, highly susceptible), the initial QTL analysis detected two QTLs, *qFSR1* and *qFSR2* , conferring resistance to *Fusarium* stalk rot. The major QTL *qFSR1* was further confirmed in the DH, F2, BC2F1, and BC3F1 populations. Within the *qFSR1* confidence interval, single/low-copy BAC sequences and anchored ESTs/IDPs were exploited to develop a total of 59 markers to saturate the *qFSR1* region. A step by step narrow down strategy was adopted in fine-mapping of *qFSR1* . Recombinants in the *qFSR1* region were screened from each BC generation and then backcrossed to 'Y331' to produce the next backcross progeny. The progeny were individually investigated for their genotypes and resistances to *Fusarium* stalk rot. Significance of the difference in resistance percentages between progeny with/without the donor regions suggests the presence of *qFSR1* in the parental recombinant, or otherwise, no *qFSR1* is present in the donor region. Comparison of phenotype and donor region among all recombinants allowed delimitation of the *qFSR1* region. After sequential fine-mapping from BC4F1 to BC6F1 generations, the *qFSR1* locus was gradually narrowed down into a ~170kb interval, flanked by the markers SSR450 and SSR477. Four candidate resistance genes were identified in the *qFSR1* locus when compared to the B73 genome sequence. We constructed a BAC library from the resistant '1145' parental line and screened four positive BAC clones that cover the *qFSR1* locus. Validation of resistance gene is conducting by isolating the candidates from the '1145' BAC clones, followed by complementary functional test. In the fine-mapping process, resistance of *qFSR1* to *Fusarium* stalk rot was also investigated from BC3F1 to BC6F1 generations. The *qFSR1* locus, once introgressed into the 'Y331' genome, could steadily enhance the resistance percentage by 32% to 43%. The *qFSR1* locus is, therefore, capable of improving maize resistance to *Fusarium* stalk rot.

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P124

A putative maize zinc-finger protein gene, ZmAN13, may participate in abiotic stress responses

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We described here a member of ZnF-AN1 gene family, ZmAN13, from maize (Zea mays), characterized by the presence of A20 and AN1 type Zinc finger domains. ZmAN13 had only one copy in the maize genome. The expression of ZmAN13 was induced by cold and abscisic acid, but not induced by NaCl and drought. At normal conditions, the expression of ZmAN13 was higher in leaves than in other organs. Promoter-GUS analysis also revealed that the expression level of ZmAN13 was higher in leaves. ZmAN13 protein fused to GFP was localized both cytoplast and nucleus in Arabidopsis protoplast and onion epidermic cells. Yeast two hybrid experiments demonstrated that the conserved A20 and AN1 domains of the ZmAN13 interact with each other in vitro. Moreover, the conserved A20 domain of the ZmAN13 interacted with itself in the yeast, otherwise the AN1 domain could not. Overexpression of ZmAN13 in Arabidopsis conferred tolerance to cold, but increased the salt and drought sensitivity at germination and seedling stage compared to the wild type Arabidopsis.

P125

A survey of transcriptional silencing of promoters by pIRs in maize and rice

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Previous work in maize demonstrated tapetum-expressed maize promoters could be transcriptionally suppressed using promoter-inverted repeats (pIRs). To determine whether transcriptional gene silencing (TGS) might be able to suppress other promoters in maize, pollen, meristem, endosperm and leaf genes were targeted by constitutively expressed pIRs while monitoring phenotypic changes. Although many of these maize promoters were effectively suppressed at high frequencies, some were resistant to silencing. Differences among promoters and pIRs were borne out further when challenged by the a suppressor of TGS, *mop2*. While *mop2* was effective at reversing silencing at tassel promoters, silencing by a pIR targeting a pollen promoter was unchanged. Recently, *mop2* has been identified as one of three genes that encodes the second largest subunit of PolIV/PolV complexes (Sidorenko *et al*. 2009). PolIV/PolV complexes are important for both siRNA biogenesis and transcription of noncoding RNAs at target loci. Moreover, Nrpd2/e2 subunits are differentially expressed in maize suggesting a complex system of biochemical and molecular components that may provide diversity to RNA–mediated transcriptional silencing at different genes and tissues. It may also be likely that regions within genes contain yet to be identified structural features that are important for recruiting these complexes and may account for the resistance to TGS across promoters by pIR based approaches. The high degree of sequence similarity between *Mop2* (Nrpd2/e2a) and its rice ortholog, *Os*Nrpd2a, suggests that both rice and maize have conserved the mechanism of transcriptional silencing. This suggestion was supported by our ability to silence rice genes using pIRs directed against promoters expressed in the panicle. Although this result differs from previous work in rice, the inability to observe silencing in those studies may be explained by the selection of promoters resistant to pIR suppression rather than lack of a TGS-mediated suppression mechanism.

P126

A virus induced gene silencing system in maize and its application to characterize maize genes involved in the *Ustilago maydis/ Zea mays* interaction

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The basidiomycetous fungus *Ustilago maydis* is a biotrophic pathogen causing corn smut disease. Transcriptome profiling at different time points after *U. maydis* infection indicated that during early infection stages *U. maydis* is recognized by the plant and elicits massive plant defense reactions which are attenuated once the biotrophic interaction has been established.

For the functional characterization of maize genes that are differentially expressed during *U. maydis* infection, a system allowing rapid, systemic gene-silencing had to be established. To this end, we adapted a *Brome mosaic virus* (BMV) based virus induced gene silencing (VIGS) system (Ding et al., 2006, Mol Plant-Microbe Interact 19: 1229) to maize at conditions that enable simultaneous infection with *U. maydis*. This system now allows parallel quantification of VIGS and its impact on *U. maydis* infection by means of a qRT-PCR based readout. Functional screening of a first set of *U. maydis* induced maize genes identified a terpene synthase being involved in control of disease development. Moreover, two proteins involved in cell death inhibition have been identified as compatibility factors that need to be expressed for successful infection of *U. maydis*.

Together, the presented screening system has potential to be a versatile tool for the rapid identification of novel maize genes that are involved in resistance to fungal pathogens.

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P127

Allelic Diversity of Two Cytosolic Glutamine Synthtase Family Members Associated with Low Nitrogen Tolerance in Maize

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Excessive nitrogen (N) fertilization with low nitrogen use efficiency in cereal crops like maize has resulted in low production efficiency along with serious environmental problems. Improving N use efficiency is thus very important for agriculture. Through an association analysis approach, two cytosolic glutamine synthetase genes (Gln1-3, Gln1-4) associated with kernel numbers and size (weight) at suboptimal N condition were analyzed among a panel of structured 160 maize inbreds with phenotyping data under contrast N input at 3 diverse locations. The haplotypes associated with kernel numbers, size (weight) and low nitrogen tolerance index, which was defined aiming at developing varieties to produce more grain yield at suboptimal N input, were found. The molecular tools might be developed based on these finding and applied into marker assisted breeding in the near future.

P128

Analysis of Gene responsible for low phosphorus stress in maize

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Phosphorus is one of the most limited nutrition factors for crop production around the world due to its important function during growth and development in plant and low availability and mobility in soils. Development of variety with high utilization of phosphorus under low phosphorus stress is the best strategy to solve this challenge. Identification of genes responsible for low phosphorus stress is thus key basis for facilitating molecular breeding in improving phosphorus utilization of new variety. In this study, QTL mapping, DNA Microarray, important homologous candidate gene reported from rice and Arabidopsis, and miRNA were analyzed under low phosphorus stress using P178 inbred line with high tolerance of low phosphorus and its derived recombination lines with 300 family lines. The preliminary results showed that 420 genes were induced and 208 repressed in response to Pi starvation and two genes encoding phytase and acid phosphatase were significantly induced by Pi deficiency and may play a big role in the process of absorption and reutilization of Pi by plants. A total of 12 miRNAs was cloned and characterized from a small RNA library constructed with maize seedling roots under low phosphorus stress, 10 of which are new in maize and two known miRNAs represent miRNA399b and miRNA156. Fifty-seven putative genes were predicted as target genes for these 12 miRNAs. In addition, two important phosphate transporter genes and few transcription factors like MYB family were cloned and confirmed by qRT-PCR. All these genes are being integrated with a linkage mapping constructed by SSR markers and further confirmed by QTL analysis.

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P129

Approaches for studying nitrogen use efficiency in maize and rice plants

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Plant adaptation to low nitrogen can be an important limiting factor in agriculture production. Using genotypes with high nitrogen use efficiency (NUE) and more adapted to lower nitrogen level will optimize fertilizer use and reduce damage to the environment. Different approaches have been used in our laboratory to understand the molecular mechanisms involved in the adaptation of rice and maize plants to a limiting nitrogen condition. Using microarray analysis, a number of genes were identified to be regulated either under low nitrogen level or during the shifting of the plants from one nitrogen level to the other. A number of the identified genes were transformed in rice and maize to test their effect on phenotype. Phenotypic and molecular analysis of the transgenic lines revealed that, some of them increase plant productivity under the limiting nitrogen condition while, other candidate genes played a role in the adaptation to general stress other than nitrogen deficiency. In addition, we have done some preliminary studies to develop methods to study maize genotypes and their adaptation to nitrogen limitation at early seedling stages. In addition to their molecular response, we used metabolic profiling to identify the key compounds involved in the plant adaptation to nitrogen deficiency. The present studies provide new avenues for exploring the physiological mechanisms underlying NUE in rice and maize.

P130

Candidate genes mining, digital gene expression profiling and co-location between minded genes and QTL for kernel oil concentration in maize

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Lipids play an important role in plants not only due to their abundance and their extensive participation in many metabolic processes but also storage stuff in grain. Genes involved in lipid metabolism have been extensively studied in Arabidopsis and other plant species. Here, we are attempting to decode the genetic architecture of oil concentration in maize kernel through bio-information methods at genome-wide level. In this study, a total of 1,003 lipid-related genes in maize were electronically cloned and annotated, including 42 genes with experimental validation, 732 genes with full-length cDNA and protein sequences in public databases and 229 newly cloned genes. Ninety-seven maize lipid-related genes with tissue-preferential expression were discovered by in silico gene expression profiling based on 1,984,483 maize Expressed Sequence Tags collected from 182 cDNA libraries. Meanwhile, 70 QTL clusters for kernel oil concentration in maize were identified, covering 34.5% of the maize genome. Fifty-nine (84%) QTL clusters were co-located with at least one lipid-related gene, and the total number of these genes amounted to 147. Interestingly, thirteen genes with kernel-preferential expression profiles fell within QTL clusters for kernel oil concentration in maize. All these results can be found at http://www.meta2trait.org. The genome-wide association based on those mined candidate lipid-related genes will be performed to discover the trait-marker associations to unravel the mechanism of oil synthesis and accumulation. All these will provide beneficial information for cloning QTL for kernel oil concentration and composition and help to better understand the molecular mechanism of oil accumulation in maize kernel.

P131

Cellular functions affected by water deficit in maize growing leaves : a proteomics approach

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Drought is one of the major environmental factors decreasing plant productivity, and it affects all levels of plant organization. The aim of this study was the identification of cellular functions that are affected by, or that participate to the response to drought in growing leaves of maize. As the leaf meristem is located at the base, there is a gradient of tissue age along the maize leaf. Thus, to study the effects of water deficit at various stages of differentiation, we analyzed leaf segments evenly sampled along leaf 6.

Plants were submitted to different water regimes: water deficit (watering arrested), water deficit followed by rewatering, and constant optimal watering (control treatment) during leaf 6's growth. The proteome of leaf segments was analyzed by 2D-electrophoresis and proteins were identified by ESI-MS/MS.

474 proteins were highly significantly affected by water regime (treatment), by differentiation or by the interaction between these two factors. 268 of them showed a significant treatment effect.

Different statistical means allowed us to characterize leaf proteome responses. The main results were as follows: most proteins involved in photosynthesis were not affected by water deficit; most chaperon-type proteins did not increase except in chloroplasts; most proteins involved in C-1 metabolism, phenylpropanoid pathway and monolignol biosynthesis decreased upon water deficit; most proteins whose quantity changed upon water deficit did not return to their control level 18h after re-watering, while leaf growth restarted. A good correlation was generally observed between protein and transcript levels. Several proteins involved in signalling or regulation showed changes in response to water deficit, and few proteins showed profiles that could be related to growth restart, either because of they returned to control levels upon re-watering, or because of their maximal accumulation in the elongation zone of control and rewatered plants. These results will be detailed and discussed.

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P132

Characterization of the antioxidant enzyme genes of glutathione-ascorbate redox cycle in Zea mays

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The enzymes and antioxidants of glutathione-ascorbate redox pathway protect cells from oxidative damage by detoxifying hydrogen peroxide. glutathione-ascorbate redox cycle include ascorbate peroxidase (APX), dehydroascorbate reductase (DHAR), monodehydroascorbate reductase (MDAR) and glutathione reductase (GR), which uses ASC, GSH, and NADPH as electron donors and catalyses hydrogen peroxide to water. Eight related proteins named ZmAPX1.1 to ZmAPX7, five ZmMDARs, three DHARs and two GRs were identified in maize. The intron-exon structure is very different even though the exon number is similar in one gene family. Among the four gene families, the most seventeen exons was found in ZmMDAR5 and the longest 11136bp intron was existed in ZmAPX4. Total eighteen genes are located on different chromosomes except for chromosome 7, and EST sequences for all the genes were identified. Subcellular location was predicted by PSORT and IPSORT programs and the results showed that these proteins are located in cytosol, mitochondria and chloroplast, respectively. The expression of these genes under different abiotic stress was investigated. APX1.1, APX2 and MDAR1 were significantly upregulated by ABA treatment with a 7-10 fold increase, and some other genes were slightly upregulated or unchanged. Interestingly, MDAR3, MDAR4 and DHAR1 were down-regulated by ABA treatment. Similar expression pattern occurred under PEG treatment condition. The expression of genes was slightly upregulated or unchanged under NaCl treatment, except that DHAR1 and DHAR2 were down-regulated. Further analysis of the promoter sequence of these genes showed the presence of ABRE element of APX1.1 promoter. The results here indicated that APX might play the major role as the first step of GSH-ASC cycle.

P133

Deep Sequencing of Small RNAs in the F1 hybrid B73 x Mo17 and its parents

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Allelic diversity and changes in dosage for key regulatory genes are hypothesized to contribute to heterosis in plants. Small RNAs (sRNAs) are a new class of regulatory factors that control plant growth and development, function in plant immunity, and maintain the structure of the genome. To assess the contribution of sRNAs to heterosis in maize, we used deep sequencing to profile the differences in sRNAs between seedling shoot apices of B73, Mo17, and their reciprocal hybrids. For this cross and this tissue, we found that miRNAs do not vary significantly in their relative expression among the genotypes. However, other classes of small RNAs did exhibit differences between the inbred parents, which are passed on to the hybrids. By far, the largest difference between inbreds and hybrids was in the siRNAs mapping to ribosomal DNA regions. For 20-24-nt rDNA-derived sRNA, hybrids had at least two-fold higher abundance. Clustering of unique sequences showed that B73 and Mo17 can differ in the 22-nt and 24-nt siRNAs generated from the same repeat element, and in the 21-nt siRNAs generated from the same cisNAT. Analysis of small interfering RNAs (siRNAs) matching the *Zea* repeats database suggests that *cinful* retrotransposons are more active in producing siRNAs in B73, whereas the *giepum* retrotransposons generate more siRNAs in Mo17. The lack of large global differences in sRNAs between inbreds and hybrids corresponds to preliminary findings that hybrids homozygous for the *MEDIATOR OF PARAMUTATION1* (*mop1-1*) mutation, which lack nearly all of their 24-nt repeat derived siRNAs due to defects in *RNA-DEPENDENT RNA POLYMERASE2* (*RDR2*) activity, do not have reduced heterosis for potential kernel number compared to isogenic wild-type hybrids. Overall, the deep sequencing results shows that patterns of differences in sRNA activity and their underlying structural basis are consistent with many of the previously described properties of heterosis.

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P134

Dissection of Genetic Basis for Kernel Tocopherols Content in Maize

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Recently, association mapping provides an important tool to dissect the genetic basis of complex traits in plants. In our lab, we have assembled an association mapping panel with 539 diverse maize inbred lines in order to identify loci controlling important agronomical traits. The first trait we focused on is kernel tocopherols content because of its high heritability, its well-known metabolic pathway and its importance as natural antioxidants and nutrients to plants and mammals. Through this study, we try to 1) assess the contribution of the well-known candidate genes to natural variations of tocopherols content in maize kernel; 2) identify other genetic loci affecting kernel tocopherols content; 3) elucidate the genetic architecture of tocopherols content in maize kernel; and 4) finally develop useful markers that could be used for high vitamin E maize breeding. The contents of three kinds of tocopherols, namely α-, γ- and δ-, were scored in a subset with 155 lines across three seasons and in the full set across one season. Based on the full set's results, the contents range from 0.48 to 59.46 μg/g for α-tocopherol, 3.08 to 150.57 μg/g for γ-tocopherol, 0.24 to 6.93 μg/g for δ-tocopherol, 4.41 to 192.70 μg/g for total tocopherols; respectively. And the ratio of α-tocopherol to γ-tocopherol varies between 0.01 and 2.48. Quantitative trait loci (QTL) analysis in three segregating populations has been carried out. Thirty-one QTL covering nine out of the 10 maize chromosomes were identified in a recombinant inbred lines population. Six genes from the tocopherols biosynthesis pathway, namely *VTE1*, *VTE2*, *VTE3*, *VTE4*, *VTE5* and *HPPD* (three of which co-located with the mapped QTL), were chosen for resequencing in the association mapping panel. One variant from *VTE4* was found to affect the ratio of α-tocopherol to γ-tocopherol significantly across all seasons in the 155 subset. In the near future, the being-developed maize 60K infinium array will be used to genotype the association mapping panel for genome-wide association study.

P135

Distributed Simple Sequence Repeat (SSR) markers for efficient mapping from maize public mutagenesis populations

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The genome sequence of the B73 maize inbred enables map-based cloning of genetic variants underlying phenotypes. In parallel to sequencing efforts, multiple public mutagenesis resources are being developed predominantly in the W22 and B73 inbreds. Efficient platforms to map mutants in these genetic backgrounds would aid molecular genetic analysis of the public resources. We screened 505 simple sequence repeat (SSR) markers for polymorphisms between the B73, Mo17, and W22 inbreds. Using common thermocycling conditions, 47.1% of the screened markers showed co-dominant polymorphisms in at least one pair of inbreds. Based on these results, we identified 85 distributed markers for mapping in all three inbred pairs. For each inbred pair, the distributed set has 64-71 polymorphic markers with a mean distance of 27-29 cM between markers. The distributed markers give nearly complete coverage of the genetic map for each inbred pair. We demonstrate the utility of the marker set for efficient placement of mutants on the maize genetic map with an example mapping experiment of a seed mutant from the UniformMu mutagenesis resource. This map position complemented transposon flanking sequence tag (FST) resources. We identified an FST corresponding to the seed mutant isolate as well as an independent allelic insertion that co-segregates with an analogous seed phenotype. These results suggest that we have likely identified the gene causing the seed phenotype. We conclude that these distributed molecular markers enable rapid mapping of phenotypic variants from public mutagenesis populations.

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P136

Divergent transcriptional responses of rice root types to arbuscular mycorrhizal colonization.

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Roots serve the plant in water and nutrient uptake and anchorage to the ground. Root systems of higher plants consist of at least two root types. Their architecture is highly plastic and strongly responsive to the environment. The rice root system is complex and consists of crown roots (CR), large lateral roots (LLRs) and fine lateral roots (FLRs). CRs and LLRs constitutively form aerenchyma and FLRs lack cortex tissue. Like many other plants rice forms arbuscular mycorrhiza, an ancient symbiosis between most land plants and fungi of the Glomeramycota. Colonization of roots by AM fungi is characterized by formation of highly branched fungal haustoria called arbuscules in the cortex where mineral nutrients are delivered to the plant. In rice the distribution of arbuscular mycorrhizal colonization within the root system is not equal. LLRs are preferentially colonized, CRs are less colonized and FLRs are neglected. This suggests that signaling events between AM fungus and root prior and during colonization and physiological changes in response to colonization might be root-type specific. To begin to understand signaling and root physiological scenarios underlying the distribution of AM colonization in rice roots, we compared whole genome transcriptional responses to AM colonization among individual rice root types. Transcriptional responses to AM colonization differed profoundly among root types with, surprisingly, strongest transcriptional changes in the less colonized CRs. PCA revealed similar expression profiles of lateral root types and a divergent profile of CRs in non-colonized root systems. However, in colonized root systems the expression profiles of CRs and LLRs were similar, while the profile of non-colonized FLRs was different. These transcription patterns indicate a shift in functional relationships among root types of a rice root stock and suggest distinct root system developmental programmes in the absence and presence of arbuscular mycorrhiza.

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P137

Evolution of meiotic recombination genes in maize and teosinte

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Meiotic recombination is a major source of genetic variation in plants. Although the role of recombination in evolution is recognized, little is known about how evolutionary forces shape the structure of the recombination pathway itself. We examined evolution patterns in eleven genes controlling key steps in the meiotic recombination pathway in a diverse set of maize inbred lines and teosinte accessions. Contrary to our expectation of highly constrained evolution, we found different selection modes in the eleven genes. Adaptive (positive) selection signatures were present in roughly half of the examined genes in maize. Interestingly, in Balsas teosinte, the closest wild relative of maize, different and fewer genes showed adaptive selection than in maize. Changes in relatively few amino acid residues were responsible for the adaptive evolution signatures. We found that several of the amino acid residues identified as targets of selection are likely to induce functional changes in their proteins. We hypothesize that the evolutionary changes in the recombination pathway may have contributed to the successful domestication of maize and its expansion to new cultivation areas.

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P138

Evolutionary relationship between thhe prolamins of maize, sorghum and sugarcane.

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Prolamins, the seed storage proteins of maize, sorghum and coix were also found in sugarcane. Prolamins are grouped into structurally distinct classes termed the α-, β-, γ- and δ-prolamins. Orthologues for almost all of the α-, β-, γ- and δ-prolamins classes were identified in sugarcane. In maize, there are two molecular weight classes of α-prolamins, the 22 and 19 kD α-zeins. Sugarcane also possesses both the 22 kD and the 19 kD α-prolamins, which we denote as caneins, whereas sorghum and coix contain only the 22 kD α-prolamin (α-kafirin and α-coixin, respectively). Amino acid sequence alignments of the 22 and 19 kD α-prolamins from these plants revealed that both the 19 kD α-zein and the 19 kD α-canein lack around 20 amino acids at the sixth α-helix domain. We postulate that the 19 kD α-prolamins originated from a deletion of the sixth α-helix of a 22 kD counterpart in the saccharum lineage. Saccharum and sorghum diverged around five to nine million years ago (Mya), when only the 22 kD α-prolamins existed. The 19 kD α-canein must therefore have emerged after this time. Sorghum possesses a 19 kD α-prolamin similar to that of sugarcane and maize, but it contains the sixth α-helix domain lacking in the 19 kD α-zein and the 19 kD α-canein. This sorghum α-prolamin that we called 19 kD-like α-kafirin must be the ancestor of the 19 kD α-canein. The 19 kD-like α-kafirin could also be the ancestor of the 19 kD α-zein but it is also possible that the genes encoding the 19 kD α-zein and the 19 kD α-canein have evolved separately in these close groups.

Funding acknowledgement: CAPES, Brazil

P139

Extensive Copy Number Variation Among Maize Lines

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Maize inbred lines show a high level of intra-specific variation. This variation can be extremely useful for development of desirable traits through plant breeding, however the specific molecular variations that underlie phenotypic variation have yet to be determined. With the recent publication of the B73 genomic sequence it is possible to address the potential role of genome structural variation in generating phenotypic diversity in maize inbreds. Prior Comparative Genomic Hybridization (CGH) analysis has identified extensive Copy Number Variations (CNVs) and Presence-Absence variations (PAVs) between the B73 and Mo17 genomes. This work was expanded to additional genotypes using custom designed 12x135k NimbleGen microarrays, based on ~32,000 genes predicted throughout the maize genome. These arrays were used for CGH analysis of 43 diverse maize inbreds and 26 teosinte genotypes. This experimental design provided the opportunity to determine the number of genes that are affected by CNV or PAV. In addition, it is possible to assess whether these represent relatively recent, rare events or events that have been present in maize haplotypes for an extended period of time. We identified several hundred genes that are present in additional copies in some inbreds and over one thousand genes that are either deleted or highly polymorphic in other maize lines. Many of these CNV or PAV events are observed in multiple maize and teosinte lines and likely represent ancient events. This information on structural genomic variation can be compiled with gene expression and phenotypic information to allow for detailed analysis regarding the underlying causes of phenotype variations within inbred lines.

P140

Fluorescent protein tagged maize lines for cell biology and functional genomics

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Differentiated maize cells consist of dynamic subcellular compartments where proteins and other components of the interactome help to maintain cellular function and order. To visualize proteins in maize cells, we are producing 100 stable lines that mark all common subcellular compartments with fluorescent tags for functional, cell biological, developmental and physiological studies. Currently, we have tagged over 70 lines, for which genomic information, clones, seeds and images are publicly available, and we have 20 at various stages of completion. We have found remarkably high stability of expression (>90% express and are stable over several generations), likely due to small transgene number, using the successful transformation strategy at the ISU Plant Transformation Facility, and also due to our tagging strategy, which requires up to 4 kb of flanking regulatory regions, plus introns, for native expression. These sequence requirements, and our prior cloning methods, has limited the tagged gene size to under 7 kb. Now, we have adapted multisite Gateway cloning methods, which permit tagging of larger genes more efficiently. Additionally, we have explored new fluorescent tags for cloning and developed new imaging technologies. With the increase in maize genome sequence and our new advances, many new candidate genes that fit our tagging criteria are now available. We will be generating more lines to complete our goal in the coming months and we welcome new gene nominations from the maize community through our project website: http://maize.jcvi.org/cellgenomics.

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P141

Future Developments for Non-Destructive 3D Plant and Root Imaging

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High-throughput screening and high-throughput phenotyping have become key technologies for research in and development of active ingredients for pharmacology, new plant protection compounds and breeding for new traits in agricultural products. These technologies are fundamentally important for many fields of applied and basic research, enabling the examination and understanding of different plant gene functions and the overall effects of chemicals on various organisms. Most of these screening methods are measuring visible parameters of the plants such as color, shape, size, area, architecture, growth rate, performance or movement. Therefore digital imaging of plants has become a very important tool in plant research, since modern image processing software algorithms are much better and more reproducible in quantifying these visual parameters than the human eye. Moreover the spectrum of modern CCD cameras can be extended to lower or higher wavelengths far beyond the visual range of the human eye such as Near Infrared (NIR) for measuring the water distribution and dynamics in plants during drought stress experiments. However all this reflective measurements are just able to target the visible part of the plant, the shoot, while the root keeps to be hidden in the soil or substrate. The goal of this joint study is, to explore weather Nuclear Magnetic Resonance Imaging (NMRI) or (Sub) Terahertz Imaging (THz) might be used for obtaining non invasive and valuable information about plant roots in soil or substrate.

P142

Genetic Architecture of Maize and Teosinte

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Genetic architecture is the constellation of gene effects and interactions that underlie variation in a quantitative trait. Essentially, genetic architecture is the map between phenotype and genotype. Understanding variation in genetic architecture is key to understanding evolution, manipulating species for a sustainable agriculture, and preserving variation as species adapt. This NSF project (DBI 0820619) will improve our understanding of the genetic architecture of complex traits in maize and its wild relative, teosinte. Maize has a combination of life history, economic and societal value, and genetic tools that make it uniquely suited to studying genetic architecture. We are identifying genes that control domestication traits and three key agronomic traits: flowering time, plant height, and kernel quality. Genetic linkage, association, and fine mapping analyses are being performed on the largest and most diverse set of mapping families publicly available for any species. A large series of isogenic lines will be used to characterize allelic series and epistatic interactions. The genetic architecture of each of the four trait groups will be compared and contrasted, and the influence of recombination and past domestication bottlenecks on the genomic distribution of functional diversity will be examined. Finally, the ability of genetic architecture-based models to predict phenotype will be evaluated in a broad range of germplasm, including elite US hybrids. This project will take a step toward the ultimate goal of predicting phenotype from genotype.

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

P143

Genetic Variability for Root Architecture in Maize

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Maize is the third most important food grain for humankind after rice and wheat. Maize is mostly grown under rain-fed conditions, and is more susceptible to drought. Both constitutive root development and adaptive root traits could be utilized to improve drought tolerance in maize. Natural genetic variation for root morphology and architecture is essential to identify valuable quantitative trait loci (QTLs) and genes contributing to root architecture. Previous mapping studies have relied on bi-parental recombinant inbred line (RIL) populations which have limited QTL resolution for complex phenotypic traits. To overcome this, McMullen et al. (2009) combined the advantages of both genetic linkage mapping and association mapping approaches. They created a nested association mapping (NAM) resource by crossing a common reference maize line, B73, with 25 diverse lines to maximize the allelic diversity that can be captured. In each cross, 200 recombinant inbred lines (RILs) were generated giving a total of 5,000 RILs available for phenotypic trait measurement. The objective of this study is to screen the 25 diverse lines and the common parental line, B73, for constitutive root traits (including rooting depth and root biomass). Initially eight lines were grown with five replications in 72 cm deep tree pots containing a turface:sand mixture (2:1 v/v) for 30 days under well-watered conditions in a temperature and humidity controlled green house. Significant variation existed among the germplasm for root length, root biomass, shoot length, and leaf area. The average root length ranged from 17.5 to 63.4 cm. The genotypes with deep root systems also recorded greater root biomass and leaf area, though there was no significant variation among the genotypes for leaf number. Screening of all the parental lines will be presented.

1. McMullen M.D., Kresovich S., Villeda H. S. et al (2009) Science, 325, 737-740.

P144

Genome-wide atlas of gene transcription through maize development from germinating seed to maturing seed

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Maize is an important model species for biological research, a major crop, and a potential biofeedstock for biofuels. One component of understanding how the underlying genetic sequence results in plant phenotype is information on the temporal and spatial transcription patterns of genes. We present a comprehensive atlas of global gene expression profiles during the life cycle of a maize plant. Based on the draft maize sequence (version 1a.49) obtained from www.maizesequence.org and additional cDNA sequences, we designed a Nimblegen gene chip containing 330,803 probes representing 66,374 gene models. We used arrays of this design to study gene expression patterns in 60 distinct tissues representing 11 major organ types of the inbred B73. We found that 59,423 (90%) of the genes were expressed in at least one tissue. This finding suggests that the current 4a.53 filtered set of 32,540 genes reported in the B73 genome does not represent all transcribed sequences. Interestingly, expression of 26,785 (40.4%) of the gene models was detected in all the tissues indicating a remarkable overlap of gene expression among biologically distinct plant organs. Despite such overlap in gene expression, principal component analysis resulted in clustering of biologically related tissues indicating unique shared transcription among related organs. This elaborated gene atlas will be an excellent resource for functional genomics and gene discovery in maize and will be made available through PLEXdb.

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P145

Genotyping by sequencing in maize

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Next generation sequencing provides a new, cost-effective means to genotype large numbers of individuals of virtually any organism at very high density. Genotyping by sequencing (GBS) can be achieved by targeting a reduced fraction of the genome such as sequence adjacent to restriction enzyme cut sites. Through the use of adapter sequence barcodes, a high degree of sample multiplexing can be attained, rendering GBS potentially far more cost effective than alternative genotyping approaches such as SNP arrays. We have demonstrated GBS in maize by genotyping 275 RILs from the IBM mapping population, filtering for sequence adjacent to *Ape*KI sites. This enzyme enriches for non-repetitive sequences in maize. The entire set of 275 RILs was genotyped on a single Illumina GA II flow cell, with a multiplex ratio of 48x in each lane. Two approaches were adopted for data analysis: (1) SNP calling via alignment to the B73 reference genome, and (2) treating each unique 64 base read (after truncation) as a dominant marker haplotype. The latter approach has general applicability, even for species lacking a reference genome. For both approaches, GBS polymorphisms were validated by genetic mapping in IBM. Hundreds of thousands of the genotype calls mapped to the expected location (with $p<0.001$), demonstrating the abundance of useful markers generated by this method. Several thousand of the GBS markers fell on "chromosome 0", a repository for contigs that have not been placed on the maize physical map. Hence, a beneficial spinoff of this work was the ability to genetically map 20 of the 30 chromosome 0 contigs to their closest SNP marker on the NAM genetic map.

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P146

Identification of genes differentially expressed in maize (*Zea mays* L.) during *Rhizoctonia Solani* Kühn infection by suppression subtractive hybridization

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Banded leaf and sheath blight (BLSB) caused by the fungal pathogen *Rhizoctonia Solani* kühn is an emerging problem in maize production worldwide. To elucidate the putative molecular basis of maize defense to the pathogen, RNA isolated from *R. solani*-infected leaves was used for suppression subtractive hybridization (SSH). After two cycles of hybridization, cloning and reverse-Northern hybridization screening, 84 cDNA clones from the forward and reverse SSH library were identified. Sequencing analysis and gene expression analysis demonstrated that these clones represent 51 single genes, and 35 ESTs sequence had significant homology with known genes including signal transduction, transcription and regulation, protein processing and destination, primary and second metabolism, defense and disease response, and other functions. The expression of fifteen differentially expressed SSH ESTs was confirmed with RT-PCR. The defense genes associated with resistance to *R. solani* identified in this study can be used for further elucidation of the response molecular mechanism to BLSB.

P147

Identification of genes related to germination by screening variability within inbreds

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Ageing reduces vigour and viability in maize inbred lines due to degenerative changes. Besides non heritable genetic changes due to chromosome aberrations and damage in DNA sequence, heritable changes during maize conservation have been reported. Genetic variability among aged seeds of inbred lines could be used for association studies with seed germination. The objective of this work was to identify genes related to germination in aged seeds. The sweet corn inbred line P39 and the field corn inbred line EP44 were used as plant material. Bulks of living and dead seeds after 20 and 22 years of storage were compared by using 226 SSRs and, when the bulks differed for a marker, the individual grains were genotyped. Differences between dead and living seeds could be explained by residual variability, spontaneous mutation, or ageing. Polymorphic SSRs between living and dead seeds were found in six known genes, including the *pathogenesis-related protein2*, *superoxide dismutase4*, *catalase3*, *opaque endosperm2*, and *metallothionein1* that were related to germination, along with *golden plant2*. Therefore, genetic variability among aged seeds of inbreds could be useful for preliminary association analysis to identify candidate genes. Seven novel candidate genes putatively involved in germination have been identified, and genetic studies to verify the involvement of two of these genes, *golden plant2* and GRMZM2G137312, in seed germination have been planned. Gene expression will be studied during seed imbibition using qPCR and candidate association analysis will check if sequence polymorphisms at those genes significantly affect variation for germination in a panel of diverse maize inbreds.

P148

Inter- and Intra-Specific Gene Expression Diversity in Maize

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Exploitation of the genetic diversity underlying phenotypic variation is fundamental for crop enhancement through plant breeding. Specifically, changes in gene expression may result in morphological changes in the plant. To better understand the effects of genetic diversity on gene expression, we have examined inter- and intra-specific divergence of gene expression in a panel of 43 diverse maize inbred lines (including 25 NAM parent lines) and 11 teosinte lines (8 inbred and 3 wild accessions). This experimental design allows us to detect genes with differential expression following domestication and to identify relationships among sub-populations within maize and teosinte. Global transcript accumulation was surveyed for multiple biological replications of each genotype using custom NimbleGen microarrays containing multiple probes for ~32,000 maize genes. With an estimated false discovery rate (FDR) cutoff of ~5%, more than 1,000 genes were detected as differentially expressed between maize and teosinte. Clustered expression patterns reveal genes with increased and decreased expression in maize relative to teosinte. In addition thousands of genes (5% FDR) exhibited differential expression in at least one phylogenetic class (stiff stalk, non-stiff stalk, tropical, etc.). In conjunction with comparative genome hybridization (CGH) data for these same lines, we can determine which genes exhibit both sequence and expression diversity to better understand their mechanisms of regulation.

P149

Is the 19KD α-prolamin gene family in maize and sugarcane a product of a convergent evolution?

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Comparison of the pattern and distribution of the seed storage protein, prolamins, of maize, sorghum and sugarcane, revealed a striking similarity between maize and sugarcane. Prolamins can be grouped into structurally distinct classes named α-, β-, γ- and δ-prolamins. Almost all classes are present in maize, sorghum and sugarcane, but in maize there are two molecular weight distinct classes of α-prolamins, the 22 KD and the 19 KD α-zeins. Sorghum possesses only the 22 KD α-kafirin while sugarcane possess both the 22KD and the 19 KD α-prolamins, which we called caneins. Alignment of the 22 and the 19 KD amino acid sequences revealed that both the 19 KD α-zein and the 19 KD α-canein lack the 6th α-helices domain from the 10 α-helices domains found in the 22 KD α-prolamins. Since the 22 KD α-prolamins are present in sorghum and in the more ancient Andropogoneae species Coix, we postulate that the 19 KD α-prolamins originated, by a deletion of the 6th α-helices domain of a 22 KD α-prolamins in the saccharum lineage after the divergence of Saccharum and sorghum occurred about 5-9 Mya. The presence of the 19KD α-prolamin in maize and sugarcane suggest that either this gene family evoluted in a convergent manner in the two species or both species had a common ancestor from which the 19KD α-prolamin originated.

Funding acknowledgement: Capes, Brazil

P150

Large-scale genome mutagenesis with the transposable element *Dissociation* (*Ds*)

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Over 1,500 *Ds* elements were distributed throughout the maize genome and positioned on the maize physical map. These insertions are displayed as tracks in the maize genome browers at PlantGDB, MaizeGDB and maizesequence.org. We here describe methodologies and pilot experiments that demonstrate usage of the collection for forward (phenotype driven) and reverse (sequence driven) genetics approaches. Given the tendency of *Ds* to transpose to closely linked sites, each element in the collection provides a launchpad to obtain insertions into nearby genes. Based on genetic experiments involving several different *Ds* donors and multiple gene targets, we derived an algorithm to predict targeting frequency as a function of the genetic distance between a donor *Ds* and a target locus. These results indicate that genes within a ± two centimorgan region flanking a *Ds* will serve as ideal targets for regional mutagenesis. Thus, with the existing set of *Ds* insertions, there is a minimum 90% probability for obtaining an insertion allele from a collection of 3,000 – 5,000 progeny, for approximately 90% of the genes in maize. For many genes, the required progeny size to score is lower. To investigate the utility of different gene knockout approaches, we compared a large number of endogenous *Ds* and *Mutator* insertion sites. These analyses revealed distinct target preferences, which indicate functional complementarity of the two elements for gene tagging in maize. In particular, *Ds* insertions display a stronger preference for insertions within exon and intron, whereas *Mutator* insertions are more enriched in promoters and 5'-UTRs. *Ds* has no strong target site consensus sequence, but we identified properties of the DNA molecule inherent to its local structure that may influence *Ds* target site selection, which could be used to enhance the specificity of targeted transpositions. Detailed information for all Ds insertion lines as well as seed stocks are available through web pages hosted in the "Datasets" submenu at PlantGDB (http://plantgdb.org/prj/AcDsTagging).

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P151

Maize Community Annotation Project to Improve Gene Structures

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High-quality maize genomic analyses require accurate prediction of all gene structures, including splicing variants (which are now estimated to occur in at least 45% of plant genes). Annotation of gene structure can be accomplished by automated pipelines that combine *ab initio* gene prediction with spliced-alignment of transcripts and predicted proteins. Human-assisted annotation has an important role in augmenting and correcting automated annotation results because humans can take into account more types of evidence than can any currently deployed pipeline. Moreover, mobilizing a research community to aid annotation has the potential to provide high quality structures for gene families of the greatest interest to researchers. The recently published draft maize genome (http://www.maizesequence.org) includes a set of automated, evidence-aided gene predictions that serves as a first look at the maize transcriptome. In order to derive maximum usefulness from this dataset for the maize research community, however, significant curation will be required. PlantGDB's yrGATE (Your Genome Annotation Tool for Eukaryotes) is an online community annotation tool for amending and/or correcting erroneous gene models, based on evidence alignment data (cDNA, EST, protein). Any user can create a Community Annotation account at PlantGDB's maize genome site ZmGDB (http://www.plantgdb.org/ZmGDB) and learn to annotate genes. As a further aid, ZmGDB automatically flags genes most in need of curation based on incongruencies with transcript evidence and publishes them to MaizeGDB's genome browser (http://www.maizegdb.org/gbrowse.php). Regions with high rice/sorghum synteny are also flagged so researchers can make cross-genome comparisons with high confidence. Using this system, MaizeGDB users can immediately identify loci in need of further annotation directly from the MaizeGDB Genome Browser and, with a few clicks, begin annotating a locus via yrGATE. All submissions are curated at PlantGDB and then published back to MaizeGDB. We will present some examples of how yrGATE can be used to improve gene structure in maize, and invite participation by the maize research community in this process.

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P152

Maize Translational Genomics for Improvement of Energy Grasses

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Grass species represent a major feedstocks for biofuel production. Most of the biomass is contributed by cell walls that are distinct in composition from all other flowering plants. Identifying cell wall-related genes and their functions underpins a fundamental understanding of growth and development in these species. Toward this goal, we are building a knowledge base of the maize genes involved in cell wall biology, their expression profiles, and the phenotypic consequences of mutation for translational genomic studies in potential energy grasses. Over 750 maize genes were annotated and assembled into gene families predicted to function in cell wall biogenesis. Comparative genomics of maize, rice, and Arabidopsis sequences reveal differences in gene family structure between grass species and a reference eudicot species. These differences in gene family structure and expression between Arabidopsis and the grasses underscore the requirement for a grass-specific genetic model for functional analyses. Application of next generation short-read sequencing protocols enhance sensitivity at least 100-fold over long read protocols. We will report expression profiles of cell wall-related genes during several developmental stages in the formation of the sclerenchyma of the maize stem. We have also employed Pyrolysis Molecular Beam MS as a high throughput means to quantify relative abundance of characteristic carbohydrate and aromatic constituents of the grass cell walls in the IBM recombinant inbred lines. We identified QTL corresponding abundance of pentose and hexose residues, acetylated xylans, hydroxycinnamic acids, and G:S lignin. We are extending this study to the NAM populations to capture the broadest diversity of maize for optimizing quantity and quality of lignocellulosic biomass that is readily translatable to other closely related energy grasses.

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P153

Maize ionomics - elemental-fingerprinting towards identification of markers with predictive value for high mycorrhizal nutrient uptake efficiency

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The majority of terrestrial plants including crops like maize interact with fungi of the genus Glomeromycota in a symbiosis called arbuscular mycorrhiza (AM). In this symbiosis both partners benefit from a bidirectional nutrient exchange at the plant/fungus interface. Fungal hyphae highly enlarge the absorbtive area of the plant root system and supply plants with essential nutrient elements like phosphorus, nitrogen, and others from distant soil regions. The mycorrhizal nutrient uptake pathway can significantly contribute to total plant nutrient uptake and thus vegetative and reproductive growth of crop plants under nutrient-limited field conditions. The maize genome contains six genes encoding phosphate transport proteins (Nagy et al., 2006). One of them, namely /ZEAma;Pht1;6/, is expressed in a mycorrhiza-specific manner in roots indicating its role in the mycorrhizal phosphate uptake pathway (Rausch et al., 2001).

The dynamic network of elements within a plant is controlled by its physiology, biochemistry and basically by the genome of a plant. Hence, elemental composition - or the "ionome" - of a plant mirrors its physiological state. The study of ionomics deals with qualitative and quantitative analysis of elements present in plant tissue.

The present study aims at the identification of characteristic elemental fingerprints in maize plants that produce high biomass under P- and/or N-depleted conditions in the presence of AM fungi. Maize B73 plants were cultivated under P-limited conditions with and without AM. Biomass production of mycorrhizal plants was increased approximately four-fold in comparison to non-mycorrhizal plants. Element composition of mycorrhizal and non-mycorrhizal plants has been comparatively analysed using the Agilent 7700 Series ICP-MS (inductively-coupled plasma mass spectrometer). In further studies natural variation in a broad spectrum of different maize lines will be used to investigate mycorrhizal nutrient acquisition efficiency (Sawers et al., 2008). Elemental composition of these plants will be correlated with their physiology and with data from transcriptomic and metabolomic analyses. This will ideally result in a global understanding of mycorrhiza-dependent vegetative growth in maize.

P154

Maximizing carbon and energy efficiencies of biofuels production

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Maize is a genetic model for all grass species envisaged to be future bioenergy crops for the production of renewable liquid transportation fuels. Second-generation biofuels will be derived from lignocellulosic biomass using biological catalysts to convert the carbon in plant cell wall polysaccharides to ethanol or other biofuels. However, for biofuels to contribute strongly to an energy portfolio independent from foreign and finite oil, C3Bio, a DOE-funded Energy Frontier Research Center, aims to develop transformational technologies that maximize the energy and carbon efficiencies of biofuels production by the rational design of both thermal and chemical conversion processes and the biomass itself. Designing new catalysts for converting lignocellulosic biomass to biofuels requires understanding the interactions of catalysts with the chemical and physical structures of the biomass at scales ranging from atoms to macromolecules. We are generating variants of cell wall structures by manipulation of endogeneous plant genes, in both Arabidopsis and maize, and novel transgenic lines that incorporate Trojan horse catalysts as the plants grow. We are also exploring thermal treatments, including fast-hydropyrolysis, that may generate a bio-crude oil suitable for catalytic upgrading. Our preliminary data show that the composition of maize stover impacts the spectrum of fragments derived from pyrolysis molecular-beam mass spectrometry (PyMBMS). This method relies on thermal degradation of the cell wall constituents under anoxic conditions to provide information about hexose and pentose content, and the content and composition of phenolic compounds derived from lignin and hydroxycinnamic acids. The fact that we can detect changes in spectral profiles from PyMBMS of different genetic lines of maize indicates that we will be likely to see differences in product distribution and behavior in fast-hydropyrolysis conditions. We hypothesize that specific alterations in the carbohydrate-lignin architecture of the cell wall will improve the efficiency of fast-hydropyrolytic and catalytic conversion. The large multidimensional data sets derived from spectroscopic and spectrometric measurements carried out in real time as thermal and/or catalytic reactions proceed need to be related to the highly complex composition of the biomass substrate. We aim to develop predictive models for the yield and selectivity of reaction products based on deep understanding of the biomass structure and the thermal and catalytic reaction mechanisms.

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

P155

Microarray analysis of differentially expressed Fusarium verticillioides-induced maize genes

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A maize oligo microarray consisting of 46,000 oligonucleotide probes (www.maizearray.org), representing >30,000 identifiable unique maize genes, was used to investigate the transcriptome patterns of the tolerant line CO441 and the susceptible line CO354 at 48 hours after inoculation with the fungus Fusarium verticillioides, the causal agent of Fusarium ear rot. The Linear Model described in Limma was used to identify probes showing significant differential gene expression in the array comparisons. In the F. verticillioides-treated seeds we observed 280 differentially accumulating maize gene transcripts compared with untreated seeds. Many of the fungus up-regulated genes found in this study were: PR proteins, heat shock proteins, resistant gene analogues, enzymes involved in the sugar metabolism, and proteins involved in the H2O2 oxidative burst, protein synthesis, folding and stabilization. To verify the microarray data, qPCR was carried out on some selected differentially expressed genes. qPCR was also performed to test the main PR genes identified in the array experiment in silks 12, 24, 48 and 72 h after F. verticillioides infection. Therefore the expression of β-tubulin and the fumonisin biosynthetic gene FUM21 was monitored to verify the presence and the activity of the fungus in the infected samples.

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P156

Mutants in dosage-dependent genes affecting seed composition or weight

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Kernel quality traits are important targets for grain improvement. Kernel quality is a complex phenotype determined in part by chemical composition and size. We identified maize seed mutants with potentially rate-limiting steps for quality traits. We screened the UniformMu transposon tagging population using a custom-built grain analyzer that collects near-infrared spectra (NIR) and seed weights from individual seeds. NIR spectroscopy is an analytical technique that reports seed composition non-destructively. Our analyzer has 100- to 1,000-fold greater throughput than other single-kernel NIR systems enabling genomics scale analysis of individual seed phenotypes. Using an automated multivariate statistical analysis workflow, 1,000 UniformMu ears segregating for *defective kernel* (*dek*) phenotypes were screened. NIR/weight data predicted *dek* heterozygous genotypes through multiple generations for 8 mutant isolates. The seed-dosage effect was further confirmed for each mutant through outcrosses to the W22 inbred. We are using next generation sequencing of transposon insertion sites to identify linked insertions to the dosage-effect loci. Successful examples of data mining strategies to identify linked insertions will be presented.

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

P157

Nucleosome Mapping and Chromatin Structure in Maize, a Novel Platform for Genome Response Assays.

(submitted by Hank Bass <bass@bio.fsu.edu>)
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Understanding the functional organization of the genome remains one of the biggest challenges in biology. A large body of research has emerged on the modifications of chromatin and their role in gene regulation. In contrast, the underlying structure of chromatin is not well characterized, despite its central importance for myriad genomic processes. The position and density of nucleosomes are primary determinants of chromatin structure. Control of nucleosome positioning is likely multifaceted, governed to varying degrees by regulatory complexes, DNA and histone modifications, ionic environment, and intrinsic DNA sequence. Knowledge of nucleosome occupancy provides an important point of reference for considering higher order structures, yet this information is essentially nonexistent in plants. To address this shortcoming, nucleosome mapping predictions in maize were made using support vector machine (SVM) software that was trained on human chromatin. Nucleosomal occupancy likelihood (NOL) plots were found to be highly informative at multiple scales of resolution. Viewed at the single-gene scale, the NOL plots revealed canonical transcription start site (TSS) "peak-trough-peak" signatures. Intriguing intron-exon boundaries signals implicate nucleosome positioning in splicing. Viewed at the BAC scale, NOL plots are ideal for genome annotation and visual scanning, pinpointing the location of genes and mobile repetitive elements. Viewed at the whole chromosome scale, NOL plots reveal expansive features that correlate with gene or retrotransposon density. Methods for nuclease sensitivity mapping using NimbleGen microarrays are also being developed and tested. These will enable large domain mapping, genome response assays, and training of SVM-based models from maize chromatin. This research is expected to provide useful annotation of the maize genome, uncover fundamental attributes of plant chromatin structure, inform testable models of nuclear architecture, and establish a new paradigm for understanding the structure of the maize genome.

Funding acknowledgement: Florida State University

P158

Organ specificity in the biotrophic interaction of maize and *Ustilago maydis*

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Infection of maize by the corn smut *Ustilago maydis* leads to formation of plant tumors, in which fungal hyphae proliferate and teliospores develop. Tumor formation can be observed in all aerial parts, however, *U. maydis* can only form tumors by penetrating host regions still active in cell proliferation. Given the fundamental differences between the various maize organs that *U. maydis* transforms into tumors, we hypothesized that the distinctive developmental changes necessary for converting maize structures to tumors would require organ-specific gene expression. Transcriptome profiling of both pathogen and host was performed using a two-organism 4 x 44K Agilent® microarray. Transcriptome data from infected seedling leaf, adult leaf, and tassel demonstrated that both plant and pathogen exhibit organ-specific expression programs. In particular, *U. maydis* genes encoding secreted effector proteins appeared to underlie organ-specific programs resulting in tumors. Reinforcing this hypothesis, *U. maydis* mutants deleted for clusters of secreted effector genes showed significant differences in virulence depending on the infected maize organ. From these results we conclude that tumor formation requires organ-specific gene expression by both partners; we speculate that a similar principle could explain the organ-specific nature of many plant-pathogen interactions. To further examine this model, *U. maydis* mutants carrying gene deletions for individual secreted proteins with organ-specific regulation are being generated and functionally tested in maize infections.

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P159

SSH-Mu-cDNA, Suppression Subtraction hybridisation as an efficient approach for the isolation of mutant-specific Mu-flanking sequence tags.

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Insertional mutagenesis is a highly efficient tool for forward genetics since the insertion eventually facilitates causal gene identification. A first problem with the use of the endogenous transposon Mutator as an insertional agent is its high copy number, since the process of validation/elimination of each individual candidate flanking sequence tag (FST) requires a considerable investment in time and resources. A second problem is its high frequency of somatic transposition.

In MuExpress, an ERA-NET Plant Biology project, work is focused on a collection of 300 maize kernel mutants bearing single gene, recessive mutations with 3:1 segregation, selected from a Mutator-induced mutant collection of 25,000 lines developed by Biogemma SAS. To simplify the FST identification/validation process we (1) isolate FSTs from cDNA rather than genomic DNA, eliminating insertions in genes not expressed in the kernel from our analysis and (2) map the mutations by genome wide SNP fingerprinting, eliminating FSTs outside the mapping interval. To reduce the capture of somatic insertions, we compare the transposon-tagged transcript displays from immature kernels originated from a wild type plant with the displays obtained from immature mutant kernels originated from two independent heterozygous plants. Finally we use a novel SSH technique to identify causal genes, which subtracts wildtype cDNA not from one but from two independent mutant samples. The result is a collection of FSTs that are common to both mutant stocks. Candidate FSTs in the subtracted material are identified in an exhaustive manner by 454 sequencing and validated by co-segregation analysis.

Funding acknowledgement: ERA-NET Plant Genomics

P160

Several approaches to clone *opaque-7*, a nutritional quality trait in maize

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There are several starchy endosperm mutants in maize that impact the nutritional quality of the grain and one of them - *opaque-2 (o2)* - was successfully used in developing Quality Protein Maize (QPM) lines. Still, *o2* remains the only recessive opaque mutant from the series of 17 that has been cloned to date. Because of their increased lysine content, these mutants can play a major role for further improving human food as well as animal feed. One of them, *opaque-7 (o7)* is an important mutant in this series, having pleiotropic effects, like *o2*. They are both thought to be regulators of the alpha-zein gene family, whose proteins make up to 70% of the total kernel protein content. Their effects are additive, while another starchy endosperm mutant – *flowry-2* – is hypostatic to *o7*. What makes *o7* unique in the opaque series is the dramatic decrease in starch content, besides the ~2 fold increase in lysine. Cloning its gene would provide us with new insight into the potential mechanisms of how its gene product regulates both protein and sugar storage in the seed. We took three different approaches in our efforts to clone this gene: 1) transposon tagging using the autonomous *Activator (Ac)* element, located at a reporter gene locus; 2) transposon tagging using a platform engineered from a *Ds(Dissociation)*-factor, mobilized by an endogenous *Ac*; and 3) map-based cloning. Mendelian frequencies are skewed and canalization of endosperm development has been reported for this mutant. Therefore choosing the right background played a critical role when working with this phenotype. The same background was used for the first two experiments as the one in which the *o7* mutant spontaneously arose, while for generating the mapping population we outcrossed it to the B73 reference genome.

Funding acknowledgement: Department of Energy (DOE)

P161

The UniformMu resource for reverse genetics in maize

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The UniformMu resource provides an enhanced reverse genetics capability to the maize community by linking Mu transposon insertions mapped in the maize genome by sequencing to publicly available seed stocks. The collection of mapped insertions (currently >7,500 unique insertions and growing) may be searched by BLAST or using the genome browser display at MaizeGDB. Through MaizeGDB, each uniquely identified insertion is linked to individual seed stocks available from the Maize Stock Center. Currently, the Stock Center seed resource includes 2451 high quality, Mu-inactive stocks. Mu-inactive (absence of MuDR) lines are selected using the bz1-mum9 marker present in every UniformMu line and seed stocks are constructed by pooling seed from sib-pollinated F3 plants. The sib-mating strategy minimizes adverse selection against deleterious mutations (often the most interesting insertions!) by maintaining them in a heterozygous state. F3 lines that pass stringent seed quality standards are grown in grid arrays (2D or 4D) analyzed by nexgen sequencing to identify unique germinal insertions. We have successfully adapted our protocol to the Illumina GSII platform affording deeper coverage of pooled DNA samples and greater statistical reliability of axis assignments.

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P162

Transcriptional gene silencing for maize hybrid production

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Transcriptional gene silencing has broad applications in plants providing a tool for studying gene function and identifying regulatory components unique to transcriptional gene control. Previous work in maize demonstrated that tapetum-expressed maize promoters can be transcriptionally suppressed using promoter-inverted repeats (pIRs) and the function of the male fertility gene, Ms45, can be complemented by transformed copies of MS45 transcribed by non-target promoters. This observation was exploited as method to control male fertility whereby the need to detassel female inbreds during hybrid production has been eliminated. Two sets of ms45 recessive inbreds can be selfed and maintained by TDNA insertions that contain a copy of the MS45 coding region expressed from one of two dissimilar non-maize promoters. These TDNAs also contain a pIR that targets the opposing non-maize promoter used to express the transformed copy of MS45 carried by the other paired inbred parent. Crossing these paired parents brings the counteracting pIR cassettes together which allows for silencing of the transformed copies of MS45. The net result of this cross yields male sterile plants due to uncovering the recessive ms45 allele carried by the parent inbreds. Fertility is restored to hybrid plants by pollination of these sterile females during production by the male inbreds homozygous for wild type Ms45. The poster will describe how this method can be applied to maintain large populations of plants that might otherwise not be competent to reproduce.

P163

Transcriptome-wide analysis of the manifestation of heterosis in young seedling roots of maize (*Zea mays* L.)

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Heterosis describes the superior vigor of F1-hybrid plants derived from crosses of genetically different homozygous inbred lines. Despite its agronomic importance, the molecular mechanisms that underlie the establishment of heterosis remain elusive. Previous studies demonstrated that heterosis in maize is manifested already during early plant development and that the superior performance of roots of F1-hybrids can be detected already few days after germination. The heterotic patterns measurable in primary roots and their fast and easy cultivation make them an ideal model to study heterosis.

Goal of this study is the analysis of molecular processes underlying the manifestation of heterosis in primary roots of *Zea mays*. Using a global gene expression analysis of two inbred lines and the two corresponding hybrids we plan i) to identify additive and non-additive expression patterns within and between gene families and ii) to determine the allelic contribution to the expression levels of individual genes. The large number of single nucleotide polymorphisms (SNPs) present in the recently sequenced maize genome and their identification by high-throughput sequencing technologies will allow for the determination of allele-specific gene expression in the hybrids. In previous gene expression analyses a specific gene – a *SUPEROXID DISMUTASE 2* (*SOD2*) – showed higher expression levels in hybrids than in the parental inbred lines (Höcker et al., 2008). Based on these findings, expression patterns of genes and gene families related to responses to oxidative stress will be analyzed in detail in order to test if the manifestation of heterosis is connected to the ability to tolerate high levels of reactive oxygen species (ROS). These analyses are complemented by enzyme activity assays performed under stress conditions. Preliminary results indicate a stronger activity of ROS-detoxifying enzymes in hybrids compared to inbred lines suggesting a role of ROS-detoxifying enzymes in the manifestation of hybrid vigour.

1. Höcker, N., Keller, B., Muthreich, N., Chollet, D., Descombes, P., Piepho, H.P. and Hochholdinger, F. (2008) Comparison of maize (Zea mays L.) F1-hybrid and parental inbred line primary root transcriptomes suggests organ-specific patterns of nonadditive gene expression and conserved expression trends. Genetics, 179, 1275-1283.

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P164

Translational research from Arabidopsis to maize

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For decades molecular research on plant growth regulation and stress tolerance mainly focused on the model plant Arabidopsis thaliana. This gathered valuable insights and has led to the identification of putative regulators for increased yield and stress tolerance of field crops. To assess how this knowledge can be translated to crops, we set up a platform for translational research to improve growth and stress tolerance in maize.

The aim of this platform is fourfold: 1. Implement maize transformation allowing testing of Arabidopsis and maize genes, 2. Develop a phenotyping platform for growth and stress tolerance and 3. Apply research strategies developed in Arabidopsis to identify regulators directly in maize and 4. Build a genomic knowledge base linking maize genes to homologs in other plant species and constructing conserved regulatory modules. The maize transformation protocol was optimized and the first lines were generated to validate the potential of promising genes from the Arabidopsis research for increased yield and stress tolerance in maize. In addition, a phenotyping platform was introduced focusing on leaf growth under control, water deprived and cold night conditions. Using kinematic analysis, the processes of cell division and cell expansion in the maize leaf can be quantified and transcriptome and metabolome studies have led to the identification of promising maize genes, which are currently under investigation. In addition, the availability of the maize genome sequence allows the development of bio-informatics tools to bridge the gap between species and make the step towards systems biology in maize.

In summary, this platform complements maize research with the vast amount of information, expertise and know-how available from the Arabidopsis research resulting in a unique way to identify new traits.

Funding acknowledgement: IOF UGent Belgium

P165

Uncovering the Sequence and Structure of Maize Abnormal Chromosome 10

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Members of Zea organize their genomes into 10 chromosomes. In a small percentage of maize and teosinte the normal chromosome 10 (N10) has been replaced with abnormal chromosome 10 (Ab10). In nature, there are three variants of Ab10. In general, Ab10 is defined cytogenetically by the addition of a terminal piece of supernumerary DNA onto the long arm of N10. The additional DNA (Ab10 haplotype) is composed of four distinct functional regions: differential segment, central euchromatin, heterochromatic knob, and distal tip. The Ab10 haplotype causes all knobs to transform into neocentromeres that visibly move poleward during cell division. Moreover, these knobs, and any linked loci, are subject to meiotic drive (preferential segregation). If heterozygous, the larger knobbed chromatid will be transmitted to progeny at rates of up to 83%. Due to its unique functions, Ab10 has been implicated as the primary cause of knob evolution, and is presumed to have had a major impact on the frequency of alleles linked to knobs. Ab10 is well understood at a genetic level; however, there is no sequence information from the Ab10 haplotype. Its precise origin and the date of its integration into Zea are also unknown. To address the problem a BAC library of a homozygous Ab10 line was created and initial screening was carried out to identify BACs from the Ab10 haplotype. With our initial sequence data the Ab10 haplotype will be mapped and dated, shedding some light on where it came from and how many rearrangements have occurred. Additionally, neocentromere and meiotic drive gene candidates can be identified.

Funding acknowledgement: University of Georgia Graduate School, UGA Plant Biology Department

P166

Zein-RFP reporter lines as tools to study 22 kD α-zein gene regulation

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Long-term divergent selection for grain protein concentration has produced populations with the known phenotypic extremes for this trait, and also illustrates the nature of responses to phenotypic selection. Mapping studies suggest that the response to selection in this experiment is dependent upon many small-effect genes. An alternative theory explored here is that the response depends on a few major regulators, which control these small-effect genes. Selection for grain protein concentration most dramatically affects the α-zeins. Of the 22 kD α-zeins, the few known regulators are OPAQUE2 (O2), the Prolamin-box Binding Factor (PBF), and factors influencing the folding of zeins into endosperm protein bodies. However, QTL studies do not show these regulators as having strong genetic effects on variation in seed protein concentration, suggesting the possibility of other key regulators of zein expression. RNA analysis and measuring protein abundance are two effective approaches for studying the regulation of zein expression, but they are also expensive, destructive, and laborious. An alternative inexpensive and nondestructive approach to investigate the regulation of zein expression is the use of zein-RFP reporter lines, courtesy of Dave Jackson's lab at Cold Spring Harbor, which have been introgressed into inbred lines derived from the Illinois Protein Strains (IPS). We found that RFP expression not only correlates with grain protein concentration, but also follows the accumulation patterns of endogenous zein genes throughout development. At all developmental stages, RFP expression was strongest in Illinois High Protein (IHP), the lowest in Illinois Low Protein (ILP) and in between in Illinois Reverse High Protein (IRHP) and Illinois Reverse Low Protein (IRLP). RFP expression was also visibly detected the earliest in IHP. Additionally, by crossing zein-RFP to an o2 mutant introgressed into IHP, we show that the activity of the 22 kD α-zeins is strongly activated by O2, further supporting the conclusion that the zein-RFP transgene reporter is regulated in the same manner as endogenous zein genes. Similar tests are being done with a transgenic line that overexpresses PBF. Future experiments will use the zein-RFP transgene as a tool for identifying regulators in ongoing genetic mapping studies.

P167

Biofortified: An educational resource for plant genetics and genetic engineering

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Biofortified is a group science blog that focuses on plant genetics, genetic engineering, and related subjects in agriculture and plant biology. To our knowledge, no other organization is making such a dedicated effort to discuss these topics on the web. The blog format allows scientists to interact with consumers in unique ways: consumers have the opportunity to ask questions and share their concerns while scientists can share their research and experience. The discussion is two-way, giving all parties the opportunity to contribute to the discussion and start topics of their own. Blog contributors can present news about recent discoveries, address misconceptions, and add discussion of political and social issues that intersect with the science. All plant modifications have risks, including traditional breeding, so we hope to encourage public dialogue that puts technology in the proper context. Biofortified will both provide consumers with science based information about genetic engineering and provide scientists with a broader perspective of the impact of their work. If we can accomplish these goals, we will have moved discussion of genetic engineering forward in a positive manner.

Funding acknowledgement: Biofortified receives financial support from its founding editors, reader donations, and a grant from Ashoka Changemakers.

P168

Maize Cell Genomics as a Springboard for Teaching Genetics and Cell Biology at a Tribal College, Little Big Horn College.

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We have been generating cell biology resources for maize functional genomics and have produced lines of maize that express fluorescent proteins. These lines are imaged using confocal microscopy to localize proteins to subcellular compartments. We have found that maize cell genomics is an ideal subject to spark students' interest in science because the lines are visually informative and compelling. Discussion of these lines promotes learning in genetics, cell biology and bioinformatics. We used these inherent elements to integrate our research into teaching workshops at a Tribal College in southern Montana, Little Big Horn College (LBHC) on the Crow Indian Reservation. Our goals were to attract students to research and to further their education at a 4-yr institution. We developed a teaching curriculum relevant to the students, which includes lectures, experiments, computer work, and field trips. The first workshop, Genetics I, is held annually at LBHC as a basic introduction to Genetics and Cell Biology. The second workshop held at UW, Molecular Genetics, focuses on learning about DNA through PCR. On average, 10 students, 2-3 LBHC faculty and 2 community members per year participate in the workshops both at LBHC and UW. Of the 40 total participating students, about 20% have pursued a higher education at universities near the reservation in MT and an additional 10% are attending or plan to attend UW. We attribute success of this program to trust promoted by communication, follow-through by the researchers, and cultural exchanges, which promote mutual respect. When visiting UW, students were presented viable options for completing their degrees at a 4-yr institution, which must include financial and family support and a robust support network. Diverse resources, including multicultural and minority support services must be leveraged to promote success. Experiences of individual students during the transition from life on the reservation to a large university town will be discussed.

Funding acknowledgement: National Science Foundation (NSF)

P169

The National Corn Growers Association: Applying Scientific Knowledge to Ensure Increasing Opportunities for Corn Growers

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The National Corn Growers Association (NCGA) is a grassroots driven trade group advocating for the 300,000 corn producers in the United States. With over 30,000 members, NCGA covers topics ranging from conservation, stewardship, trade, public policy, biotechnology, renewable fuels and research. Currently, we have 48 state checkoff and grower groups active within NCGA driving our priorities and keeping us focused on the challenges and opportunities facing U.S. producers.

NCGA spearheaded the formation of the National Plant Genome Initiative (NPGI) funded at $40 million dollars per year. We have since grown that line item to over $100 million per year, which has yielded the high quality corn genome sequence as a result. NCGA has remained very active in the development of the program, participating in numerous stakeholder meetings to ensure that the focus of the NPGI remains on innovation in economically important plants. We are excited about the possibilities for the maize research community to meet many of the objectives set forth by the NPGI.

Additionally, NCGA has long advocated for the development of the National Institute of Food and Agriculture (NIFA) to supplement USDA and NSF research agencies. We continue to support the development of NIFA such that it will have the capacity and funding to attract the best scientists to conduct cutting-edge research, education and extension, and to train future agricultural scientists to meet the world's food, feed and fuel needs.

Of course, the future success of agricultural research will be highly dependent on the availability of adequate funding. NCGA will continue to fight for higher levels of research funding and needs the help of both public and private sector scientists to make us successful.

P170

QTL mapping of a factor upstream of *teosinte branched1 (tb1)* that effects ear morphology

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The domestication of maize has resulted in striking morphological differences between maize and its wild progenitor, teosinte. These dramatic differences are partially controlled by the gene *teosinte branched1 (tb1)*. Our work is focused on studying a *cis* regulatory region located ~58-69 kb upstream of *tb1*. A mixed model in SAS was used to analyze six years of phenotyping data, including 28 different introgression lines containing recombination events in the regulatory region previously described. This analysis provides evidence for multiple regulatory elements in the ~58-69kb region. These regulatory elements contribute independently to control plant architecture and inflorescence morphology. To identify additional linked factors located further upstream of *tb1* a different population of Recombinant Inbred Lines (RILs), containing a larger introgressed segment of teosinte, was used in a Quantitative Trait Loci (QTL) mapping study. Imputation mapping of QTL, using R/QTL, identified a QTL which co-localizes with the previously identified regulatory region for all traits. A second QTL was identified for ear morphology traits and is located approximately 4Mb upstream of *tb1*. Our results provide evidence for a statistical interaction between these two QTL. Fine mapping and candidate gene exploration of the QTL region are being pursued. By studying the regulation of *tb1* we hope to use the domestication of maize as a model to better understand the evolution of regulatory elements controlling complex traits.

Funding acknowledgement: United States Department of Agriculture (USDA)

P171

Segregation Distortion Results in a Novel Approach to Identify Grain Yield QTLs

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Segregation distortion is a fairly common occurrence in most mapping populations (Lu et al., 2002). However, in most cases, both alleles are represented in the population and it is possible to examine the contribution of the skewed alleles to the phenotype of interest. One of the mapping populations that our lab is using to study grain yield exhibited an extreme version of segregation distortion, namely only one of the alleles was transmitted to the progeny. This particular mapping population was generated from a sister-line cross of CG60 and CG108. Previously we had shown that these lines were ~70% identical-by-descent, having come from the same bi-parental cross (Lee et al., 2006). What we observed in this nearly identical Recombinant Inbred Line population (niRIL) was that several genomic regions from CG108 were not present in the niRIL population. Across 24 hybrid yield trials, CG108 exhibited a level of performance and stability not observed in any of the niRILs. Even niRILs that contained large proportions of CG108 tended to perform similarly to CG60, suggesting that one or more of these distorted regions may control a large proportion of phenotypic variation for grain yield. The 2 major linkage blocks that were distorted involved the short arms of chromosomes 8 (8S) and 9 (9S). In an attempt to examine the contribution of the CG108 8S and 9S regions to grain yield, we selected 5 niRILs that were mostly CG108 (i.e., 80-82% CG108 by descent). These 5 niRILs were reciprocally crossed to CG108; the 10 F1s were self pollinated; and equal numbers of F2 plants from each F1 were self-pollinated to form F2:F3 lines (8S/9S niRILs) that were ~96% homozygous. From a set of 188 8S/9S niRILs, we selected 84 8S/9S niRILs that were homozygous for either the CG60 or CG108 alleles in these regions. These selected 8S/9S niRILs were testcrossed and evaluated in hybrid yield trials using the same tester that was used in the original niRIL population.

1. Lee EA, Singh A, Ash MJ, Good B (2006) Use of sister-lines and performance of modified single cross maize hybrids. Crop Sci 46:312-320
2. Lu H, Romero-Severson J, Bernardo R (2002) Chromosomal regions associated with segregation distortion in maize. Theor Appl Genet 105:622-628

P172

A 50K SNP Infinium chip for maize genome analysis

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In a collaborative effort between multiple partners (Illumina, USDA, Syngenta, INRA and TraitGenetics), an Infinium array has been designed with more than 50000 maize SNPs. This array contains SNPs in approximately two-thirds of all maize genes and additional SNPs spread over most of the remaining maize genome resulting in an average marker density of approximately one marker every 40 kb. In pilot experiments, the array was used to study the quality of the used SNPs in a set of maize germplasm. It was found that the SNP call rate in maize is lower than in other organisms. Major reasons for that are the high level of genetic diversity identified between different maize lines and associated with that the high frequency of SNPs. Data regarding the level of polymorphism in mapping populations and mainly European breeding material will be presented.

P173

A Very High Volume, Fast and Flexible Genotyping Platform for Marker Assisted Breeding

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The use of molecular markers in breeding programs is an essential tool for the rapid and simultaneous introgression of simple as well as complex traits into many field crops such as maize. One of the greatest challenges for a marker assisted plant breeding program, is the timely and precise delivery of genetic information to the breeders and geneticists. Tissue samples are routinely screened for projects based on transplanting deadlines or other time sensitive criteria, and millions of data points are to be generated and reported on a weekly basis, for successful advancement to the next generation. In order to meet this challenge, the deployment of high throughput genotyping platforms is critical. One such platform is based on less than 1 ul PCR reaction volume with in-line distribution of reagents into plastic interconnected supports, referred to as Array Tape™. Current fluorescent SNP chemistry can be easily adapted to the Array Tape™. We will show the benefit of this genotyping platform for diverse maize breeding applications. The small volume PCR combined with the Array Tape™ technology is a very high throughput, cost efficient, and flexible SNP platform that is very well suited to high volume commercial applications in marker assisted breeding.

P174

A major QTL on bin 1.06 controls root and agronomic traits in maize across water regimes

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Previous studies carried out in maize using a population of F2:3 families derived from the cross Lo1016 × Lo964 evidenced a major QTL in bin 1.06 affecting root and shoot traits in hydroponics as well as adult plant root and agronomic traits under both well-watered (WW) and water-stressed field conditions (WS). Starting from two different F2:3 families, we developed two pairs of near isogenic lines (NILs, as F7:8) at this QTL. The objective of this study was to evaluate the effects of the 1.06 QTL at varying water regimes, genetic backgrounds and inbreeding levels. NILs were evaluated per se and in hybrid combination with related testers Lo1016 and Lo964 under WW and WS conditions for two years. The NILs per se evidenced a marked decrease under WS, as compared to WW, especially for grain yield (-60%). The interactions QTL × irrigation treatment and QTL × family were negligible for most traits. The QTL additive effect across families was significant for several agronomic traits, especially root clump weight. For NILs'crosses, the WS treatment affected negatively several traits, as compared with WW, though less severely than for NILs per se (for grain yield, -29%). The interactions involving the QTL effects were negligible for most traits. The QTL additive effect across irrigation treatments and families was significant for several traits and consistent with the additive effect of NILs per se (in all cases the plus allele was from Lo1016); the QTL dominance effect was significant especially for grain yield. Collectively these results suggest that the QTL acts mainly constitutively, rather than being water-stress responsive (at least within the range of water stresses herein investigated) and its effect is not much influenced by epistasic interactions. Moreover, a concurrent effect of this QTL on plant size, overall vigour and grain yield can be hypothesized.

P175

Artificial Selection and the Genome: "Deep Pedigree" Analysis in an Elite Soybean Cultivar

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How does the practice of long-term artificial selection shape a plant's genome? Unfortunately this is one question that cannot be easily addressed in maize, as the existence of "deep pedigrees" in public sector maize breeding programs is rare. However, soybean [Glycine max (L.) Merr.] breeding programs have been routinely practicing pedigree breeding for nearly a century, making them an excellent source of genetic material for answering this question. OAC Bayfield was a highly successful commercial cultivar developed by the University of Guelph's soybean breeding program and released in 1993. Among elite soybean cultivars, OAC Bayfield stands out because of its high yield stability and longevity in the market place. Because of these unique characteristics, it has been used as a parental line for a number of successful next-generation cultivars. The development of OAC Bayfield represents 80 years of plant breeding activity in Canada. Because of its exceptional performance as a cultivar and its presence in the pedigrees of many new cultivars, the pedigree of OAC Bayfield is an excellent candidate to examine changes to the genome as a result of long-term artificial selection. In total 5-6 generations of cultivars comprising the pedigree of OAC Bayfield as well as 2 generations of cultivars developed from OAC Bayfield were genotyped with SSR markers across the genome to investigate genetic diversity, identity-by-descent estimates, linkage block transmission and other genetic parameters related to plant breeding theory. The power of "deep pedigree" analysis and the effects of long-term artificial selection on shaping the genome of an elite cultivar will be discussed.

P176

Association and linkage analysis implicates the *Ts2* region in resistance to NLB in maize.

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Northern Leaf Blight (NLB) is a foliar fungal disease of worldwide importance in maize. Resistance to NLB is conditioned by several major (qualitative) genes, as well as a host of minor (quantitative) genes. The quantitative resistance genes provide the most promising aspect for long-term sustainable control of NLB. A maize diversity panel comprised of 300 inbred lines was screened for NLB in multiple environments. Association analysis using 4,000 SNPs identified several putatively significant SNPs, including one located in the *Ts2* gene in bin 1.03 in the maize genome. The *Ts2* gene encodes the last step of a jasmonic acid pathway that leads to pistil abortion during tassel development. In this study, we report mounting evidence of a role in the *Ts2* region in defense response. A previous QTL study has implicated a QTL at bin 1.03 in maize. While no QTLs were detected in the large Nested Association Mapping population in bin 1.03, one of the sub-RIL populations had a QTL in the *Ts2* region when analyzed individually. An SSR at this gene was found to be under selection in a recurrent selection population for NLB, and two unique populations derived from independent lines from the last cycle of selection had a significant QTL at the *Ts2* locus. We are characterizing and analyzing the sequence variation of the *Ts2* region in maize to determine the putative role of the *Ts2* region in defense response.

P177

Association genetics investigation of flowering time in a panel of American and European maize inbred lines

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Maize has proven able to adapt to a wide range of altitudes and latitudes by adjusting flowering time to local environmental constraints. Numerous studies that have mapped Quantitative Trait Loci (QTL) for flowering time have been synthesized in comprehensive recent meta-analyses, showing that more than 60 QTLs contribute to the variation of flowering time, out of which some display a relatively large contribution in various genetic backgrounds. In parallel, association genetic strategies were developed to investigate a wider range of diversity and to finer decipher the genetic architecture of flowering time. We summarize here the characteristics of genetic diversity organization within a panel assembling 375 inbred lines representative of diverse European and American origins and their consequences for association genetics. We focus on the complementarities between linkage based fine mapping and association genetics and compare the effect of allelic series for two case studies that are the major QTL *Vgt1* and another major QTL recently fine mapped in our group on chromosome 10. We then illustrate how the analysis of polymorphism in a highly diverse set of worldwide open pollinated population varieties brings further elements to the understanding of adaptation.

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P178

Association study in Japanese rice population

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To utilize diverse phenotype and useful variants of agronomic traits in genetic resources, association study is an efficient way to map and identify genes/QTLs without crossing population. Association study is to detect the genetic association between DNA polymorphism (e. g. single nucleotide polymorphism (SNP) and insertion/deletion) and the phenotype of individuals and lines in a natural population. Since rice is a highly selfing species, cultivars and accessions can be genotyped once and phenotyped repeatedly. A major problem with rice association study is high level of population structure in a given population. Following the original rule for association study we used the Japanese rice population composed of 114 cultivars, which could assume that these cultivars were related from an ancestral group. We conducted association study in a specific gene region as an example of *Semidwarf1* and genome-wide association study (GWAS) of days to heading. Population structure analysis revealed six subgroups in the Japanese rice population. Therefore, mixed-model and Bayesian-model accounting for population structure and kinship were applied for our association study. For association study of *Semidwarf1*, functional SNP for the *semidwarf1* variety Jukkoku were associated with traits related to culm length in multiple environments. The GWAS of days to heading with large number of SNPs and SSRs was able to identify the known major QTLs, demonstrating the potential of rice GWAS. The GWAS simultaneously detected promising new associations for days to heading. Association study in rice is feasible upon due consideration of study design. We will discuss the association study in rice as compared with maize.

Funding acknowledgement: Ministry of Agriculture, Forestry and Fisheries of Japan (Genomics for Agricultural Innovation, NVR-0002)

P179

BIORES* PROJECT: Study of modified b-32 RIP maize proteins in plant protection against pathogens

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One of the main topics of maize breeding is the improvement of plant protection against pathogens. Plants respond to attack by pathogenic fungi with a complex network of active responses such as the production and accumulation of proteins that are toxic or inhibitory to pathogens such as RIP (Ribosome Inactivating Protein). The role of RIP in the pathogens defense has been documented. In maize endosperm, a cytosolic albumin termed b-32 is synthesized in temporal and quantitative coordination with the deposition of storage proteins. In the past years b-32 was shown i) to enzymatically inactive ribosomes modifying rRNA inhibiting protein synthesis in vitro ii) to inhibit the growth of Rhizoctonia solani mycelia in an in vitro bioassay and plant assays. In this context, we have recently shown and that maize b-32 was effective in wheat transgenic lines as an anti fungal protein by reducing Fusarium culmorum head blight (FHB) (Balconi et al., European Plant Pathology 117: 129-140, 2007) and in maize transgenic lines reducing Fusarium verticillioides attack symptoms in leaf tissues assays (Lanzanova et al., European Journal of Plant Pathol. 124: 471-482, 2009). Similarly to other RIPs, maize RIP is accumulated in the seed as an inactive precursor, which is converted into an active form by proteolytic processing which removes peptide segments from the N (residues 1-16 of pro-RIP) and C (residues 295-301) termini and also from the center of the polypeptide (residues 162-186). Aims of the BIORES project are devoted to deepen our knowledge about relationships between structure and substrate specificity of b-32 protein, in order to clarify the role of the processed segments of b-32 gene on the ability of maize RIP to inhibit fungal growth. Thereby, a series of genetic constructions was made by selectively deleting the N-terminal, or C-terminal or internal linker domain. Genetic deletions of the b-32 gene, that is apparently responsible for suppressing enzymatic activity in the precursor, will be primarily expressed in Escherichia coli to produce sufficient quantities of modified proteins. To assess the role of bioactive b-32 modified protein protection against fungal pathogens (F. verticillioides, Aspergillus flavus), a series of in vitro bioassay will be performed to analyze their effect on the fungal growth and on mycotoxins accumulation.

P180

Breeding of chilling tolerant energy maize

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Chilling tolerance is an important prerequisite for cultivation of high-yielding energy maize in central and northern Europe. In previous work, we identified QTLs for chilling tolerance in a double-haploid population generated from a cross of the chilling-sensitive parent SL with the chilling-tolerant line TH. Two major QTLs explaining 33.7 and 13.9 % of the phenotypic variation were mapped to chromosomes 4 and 5, respectively. These QTLs were validated by a phenotypic analysis of Near Isogenic Lines (NILs) carrying the chilling-tolerant allele from TH in the genetic background of the sensitive line (SL). We now aim to characterize the QTLs, to identify the genes involved in the manifestation of chilling tolerance and to understand the molecular basis of chilling tolerance.

To enrich the QTL regions with molecular markers we take advantages of both, the recently published maize genome sequence and the available physical map of maize. To narrow down the QTLs new subNILs are being produced and tested in the field. These genetic approaches are complemented by an analysis of the global gene expression pattern in response to chilling. Initially, transcriptome profiles of the sensitive parental line and tolerant NILs have been investigated using Affymetrix Maize 18K arrays. The two genotypes revealed a very similar gene expression patterns in response to chilling with just 0.5% of the genes represented on the array being differentially expressed between the genotypes. Now the 454 sequencing technology is applied for a deeper characterization of inter-genotype transcriptome differences.

Funding acknowledgement: Federal Ministry of Education and Research (BMBF, Germany), KWS SAAT AG (Einbeck, Germany)

P181

Breeding procedures at the level of haploid sporophyte in maize

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Germplasm estimation. To estimate the contribution of allelic gene interactions versus non-allelic gene effects in the development of valuable traits, thirty synthetic populations (Iowa State University) have been tested at both the diploid and haploid levels. A negative correlation between grain yield in the diploid populations and ear parameters at the haploid level has been revealed. However, there were populations with a good performance at the both ploidy levels, which have been characterized as a source of favorable genes with non-allelic effects.

Selection. After four cycles of 'Haploid Recurrent Selection' in two synthetic populations an average gain per cycle for grain yield was more than 10% raising the performance of the populations up to the level of check hybrids. Gains per cycle for quantitative traits in the diploid populations were very close to those obtained at the haploid level.

Heterosis. About 15% of haploids, derived from two F1 hybrids, significantly exceeded the haploids of the best parent line for plant height and ear length. Based on the distribution of these traits, the following assumptions have been made: in two of four cases, the transgressive effect was caused by the additive actions of genes; in other cases, there were the epistatic interactions of genes.

P182

Combining two stresses creates a joint-stress environment that has a different genetic architecture

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In the field multiple stress conditions often co-occur. Plant breeders typically use a variety of test sites (environments) to select for genotypes that have little environmental fluctuation, or to correlate measured climate data with plant performance. However, controlled physiological experiments rarely consider more than one stress factor. In soybean, pea and wheat, combined drought and ultraviolet radiation show less effect than predicted from single stress treatments. The simplest explanation of this synergy is that alleles have opposite effects and thus cancel under joint stress conditions. Recent analyses of large-scale changes in gene expression have suggested that the pattern of response to a combination of stresses is not always predictable from the pattern of expression of individual stresses. In contrast to the physiological experiments, the expression data suggest that genetic control of adaptations to multiple stresses is distinct from individual control and implies that different controlling loci would be detected in mapping experiments for joint versus single stresses.

Predicting allele by environment interaction is key in understanding the non-linear functions relating gene states to phenotypes, and in addressing the question of 'what is a different environment from a plant's perspective?'. We focus on a simple and relevant controlled environment case, examination of the effect of combining drought and ultraviolet radiation stress treatments on the genetic architecture in the IBM and NAM mapping populations. Our QTL analysis has shown that a joint-stress environment is not a simple combination of single-stress allele effects; the presence of a QTL in one stress is not a good predictor of the importance of that locus in a combined-stress environment.

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P183

Comparison of QTL detection in intermated and conventional maize recombinant populations derived from a Flint x Dent F1 hybrid.

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Advanced intermated populations have been proposed as a way to increase the accuracy of QTL mapping experiments. A conventional F3 population of 300 lines and an advanced intermated F3 population of 322 lines (called LHRF-F3), both derived from parental maize inbred lines F2 (early flint) and F252 (early dent), were jointly evaluated in testcross progeny for dry grain yield, grain moisture and silking date. Genetic variance for dry grain yield was significantly lower in LHRF-F3 than in the conventional population. A total of 30 and 21 QTLs were detected for the conventional and intermated populations, respectively. A reduction in QTL confidence interval was observed in LHRF-F3 with an average factor of 2.31. One QTL for dry grain yield detected in the conventional population was split into two QTLs in coupling phase in LHRF-F3 population. However, fewer QTLs were detected in LHRF-F3 and less than 50% of the detected QTLs were common between the two populations. Cross-validation showed that selection bias was more important in the LHRF-F3 population and that each QTL explained a lower percentage of the variance. This supports the hypothesis that the actual number of QTLs involved in the genetic architecture of the studied traits is substantially larger than the one inferred from conventional populations with usual population sizes. Fixed lines of the intermated populations are currently analyzed to complete mapping for markers monomorphic in the IBM population.

Funding acknowledgement: Genoplante

P184

Correlation of parental transcriptome and field data for the characterization and prediction of heterosis in maize

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Heterosis is widely exploited in plant breeding, although its molecular basis is still not fully understood. For the characterization of the phenomenon and the development of transcriptome-based methods to predict hybrid performance, we profiled 21 European maize (14 dent and 7 flint) parental inbred lines at the seedling stage with the 46k oligo microarray (www.maizearray.org). Based on these transcriptional profiles we investigated distance measures and compared them with genetic distances based on AFLP markers to assess their suitability for grouping of germplasm.

Hybrid performance for grain yield and grain dry matter content as well as heterosis of the corresponding 98 hybrids from flint x dent factorial crosses were assessed in field trials at six locations. We observed highly significant correlations of the parental expression levels of certain differentially expressed genes with heterosis and hybrid performance for the two traits, indicating a prediction potential of the genes and their expression levels. Cross validation showed that prediction of hybrid performance with transcriptome-based distances using selected markers was more precise than earlier prediction models using DNA markers or general combining ability estimates using field data. Our results suggest that transcriptome-based prediction of hybrid performance and heterosis has a great potential to improve the efficiency of maize hybrid breeding programs.

Additionally, the identified gene sets revealed functional characteristics of hybrid performance and heterosis by Gene Ontology analyses. The phenomenon of heterosis was characterized by the over- and underrepresentation of specific GO terms among heterosis-correlated genes. Among grain yield and grain dry matter content mostly different functional classes were found to be enriched or underrepresented.

Funding acknowledgement: German Science Foundation

P185

Diallel analysis of elite EX-PVP commercial inbreds

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Within the last half century, elite inbred and hybrid development has shifted from the public sector to the private seed industry. Seed companies have devoted substantial resources over many decades to improve their germplasm through informed crossing and intense selection. The Plant Variety Protection Act of 1970 provided companies with a means of protecting their best inbreds, which led to different germplasm pools within each company. Recently, certain elite lines frequently used in the production of subsequent elite inbreds developed by Pioneer Hi-Bred and Holdens Foundation Seeds were released from Plant Variety Protection (PVP). The expiration of patent protection represents a unique opportunity to evaluate genotypic and phenotypic variation observed in hybrids from the two different pools of germplasm. Tweleve inbreds representing key founders in the lineage of contemporary commercial U.S. Midwest corn hybrids were mated in a half-diallel crossing design to produce 66 F_1 hybrids and 66 F_2 populations from selfing the hybrids. The diallel design allows estimation of genetic effects for additive, dominance, general combining heterosis, specific combining ability, and additive by additive specific epistasis. Results from these analyses for a host of traits, including grain yield and number of days to flowering, will be presented. This diallel is part of a larger effort to create a Hybrid Association Mapping Panel which will facilitate the investigation of effects of candidate genes on hybrid vigor.

Funding acknowledgement: Dow Agrosciences

P186

Detection on genetic integrity of conserved maize germplasm in genebank using SNP markers

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The genetic integrity of twenty maize accessions conserved in different cycles from five genebanks was investigated by 1150 Single Nucleotide Polymorphisms (SNPs) and 235 SNP haplotypes. The genetic diversity of three accessions changed significantly in terms of average allele number per locus. The majority of loci had major allelic frequency (MAF) changes less than 0.05 after regenerations in accessions from all the five genebanks. Ten out of twenty accessions investigated showed significantly different allelic frequencies after regenerations, but the proportion of loci with significant allelic frequency change was very low. And for SNP haplotypes, the genetic diversity of four accessions changed significantly in terms of average allele number and polymorphic information content (PIC) per locus. In addition, the proportion of alleles lost in the regenerated cycles ranged between 0 and 22.6%, and at the same time 0-19.9% of the alleles were detected in the regenerated cycle but absent in the original cycle. The loss of genetic integrity experienced a continuous process and can be caused by several reasons like genetic drift, selection and handling errors etc. The information investigated by molecular characterization combined with the knowledge of historical documentation during regeneration is beneficial to the practice of genetic resource conservation.

Funding acknowledgement: The Consultative Group on International Agricultural Research (CGIAR), System-wide Genetic Resources Programme (SGRP)

P187

Differential zein protein levels in a *su1* population divergently selected for visual endosperm starchiness

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The *Sugary1 (Su1)* locus encodes for a starch debranching enzyme with isoamylase-type activity. The homozygous recessive state *(su1/su1)* results in the accumulation of phytoglycogen at the expense of starch. Divergent recurrent selection for visual endosperm appearance was implemented in a population that was homozygous *su1*. Six cycles of selection for extreme sugary (esu) and extreme starchy (pseudostarchy) (psu) endosperm appearance was performed. A preliminary screening of the zein profiles of the base population (cycle 0), and cycles 2, 4, and 6 of selection for both the esu and psu traits was conducted. HPLC analysis was used to determine whether differences in zein content accompanied visual endosperm selection for starch content. Differential zein accumulation was observed over cycles of selection and between esu and psu populations. A comparison of inbreds derived from cycle 6 extreme sugary (C6esu) and cycle 6 pseudostarchy (C6psu) populations supported HPLC trace specificity at the population level with even greater differentiation. Additional experiments to further characterize the zein profiles are in progress. Endosperm mRNA Transcript levels harvested at four day intervals from 2 to 18 days after pollination (DAP) from C6esu and C6psu were examined using maize spotted micro-arrays. Unique time course transcriptional profiles involving starch and protein synthesis were resolved between C6esu and C6psu. All nine classes of genes encoding zein storage proteins had significant differential expression at specific time points and six had parallel expression patterns with up regulation in C6psu at 18 DAP. No genes encoding zein proteins were consistently up or down regulated across time in either C6esu nor C6psu. However, when all zein encoding features were averaged over DAP, zein transcription was significantly up regulated in psu endosperm.

Funding acknowledgement: American Seed Research Foundation, University of Wisconsin-Madison College of Agricultural and Life Sciences, Hatch Funds

P188

Dissecting Natural Diversity in Maize Plant Height: GWAS and Fine Mapping of Chr9L QTL

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Plant height represents one of the most canonical complex traits and remains a principal determinant of light capture, carbon sequestration, and nutrient utilization. Mapping natural variation for maize plant height is therefore beneficial in breeding for improved grain, silage, and biomass yields and in elucidating the molecular underpinnings of any selection gains to be made. Using allele series defined by joint-population linkage analysis of plant height in the Nested Association Mapping population (NAM), we implemented genome wide association mapping (GWAS) across the panel of 27 diverse inbreds (NAM founders) used to construct a first-generation haplotype map of maize. Additionally, efforts to mendelize two of the largest effect plant height QTL within NAM germplasm are underway. Analysis of near isogenic lines (NILs) possessing complementary tropical introgressions (CML277, CML333 in a B73 background) on the long arm of chromosome 9 reveal significant increases in plant and ear height relative to B73. Despite tropicalness of the donor lines, no pleiotropy with flowering time was identified at the plant height loci by either joint-population analysis across NAM or comparison of the NILs.

Funding acknowledgement: United States Department of Agriculture (USDA)

P189

Ear secondary traits related to aflatoxin accumulation in commercial maize hybrids under artificial field inoculation.*

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The availability of reliable methods for screening maize genotypes for tolerance to Aspergillus, is an unvaluable tool in breeding programmes. The aims of the research were: i) to evaluate 24 maize hybrids for Aspergillus flavus resistance and for aflatoxin accumulation under artificial inoculation in field trials, during 2005 and 2006, at the Experimental Station- CRA-MAC ii) to estimate the relationship of aflatoxin content with ear secondary traits.

Primary ears were inoculated with spore suspension (five A. flavus isolates from Northern Italy), by spraying silks; controls were noninoculated and sterile water-inoculated ears. Ear secondary traits, such as silk channel length at pollination and husk coverage at maturity (rating from 1-good- to 5-poor-), were recorded. The severity of ear A. flavus rot was evaluated rating the percentage of kernels with visible symptoms of infection. Environmental conditions were recorded at the Weather-Station CRA-MAC.

Aflatoxin content in the inoculated ears resulted, during both years, higher than in the controls. Variability was found among the genotypes in aflatoxin accumulation, after artificial inoculation. Silk channel length recorded at pollination resulted significantly negatively correlated with aflatoxin accumulation; on the other hand, a positive significant correlation between husk coverage rating at maturity and aflatoxin content has emerged to suggest that a looser husk coverage is associated with higher aflatoxin accumulation. The correlation between the two ear traits was significantly negative suggesting that hybrids showing a good coverage at pollination stage, could be favoured in keeping the ear tip covered until maturity, reducing the risk of aflatoxin contamination. The above mentioned ear secondary traits could be used in breeding programs devoted to increase maize aflatoxin accumulation resistance.
*Research developed in the Research Program AFLARID, Italian Ministry of Agriculture, Rome, Italy

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P190

Effect of population structure corrections on the results of association mapping tests in complex maize diversity panels

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Linkage disequilibrium mapping of sequence polymorphisms underlying the phenotypic variability of quantitative agronomical traits has become a widely used method in plant genetics. However, due to the common presence of a complex genetic structure within the plant diversity panels, spurious associations are expected to be highly frequent. Several methods have thus been suggested to control these panel structures. They mainly rely on ad hoc criteria for selecting the number of ancestral groups; nevertheless, such group choice is not evident in the complex panels that are commonly observed in maize. We thus evaluated the effect of the selected structure models on the association mapping results. A real maize data set (342 maize line and 12 000 SNPs) was used to carry out this study. Our panel structure was estimated using both Bayesian and dimensional reduction methods and considering an increasing number of ancestral groups. Our results clearly show a significant effect of the selected structure model in the association mapping tests. This effect depends particularly on the number of ancestral groups and suggests that the structure models to use vary according to the trait analyzed. The less a trait is correlated to the panel structure the fewer number of ancestral groups is necessary to control the false positives. Our results also show that too many ancestral groups leads to an over-corrected model in which all the causal loci vanish.

P191

Field Evaluation and Genetic Characterization of a Quantitative Trait Locus conferring Resistance to Southern Leaf Blight

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Southern leaf blight (SLB) caused by the fungal pathogen *Cochliobolus heterostrophus* (anamorph = *Bipolaris maydis*), is a common disease of maize in southeastern US, as well as many hot and humid tropical and subtropical areas in the world. Most of the disease resistance used in maize is quantitative in nature; however, quantitative disease resistance remains poorly understood. To investigate quantitative resistance to SLB, we used the highly resistant inbred maize line NC292. This line is derived from crossing NC250, an elite source of SLB resistance, to the highly susceptible line B73 followed by three further backcrosses to B73 and several rounds of selfing. At each stage in this process the plants were selected for SLB resistance. Using genome-wide marker analysis of NC292, we detected 12 NC250-specific introgressions. Furthermore, 9 disease QTLs associated with SLB resistance were mapped on a related population, from which 4 colocalized with NC250-specific introgressions. We identified a strong QTL for SLB resistance at the tip of the short arm of chromosome 6 of maize which colocalized with a NC250 introgression in NC292, namely introgression 6A. Specific objectives of this research include fine-mapping introgression 6A, cloning of the gene that accounts for this effect, and evaluating yield and fitness effects of this introgression under both high and low disease pressure for possible future use of this resistance. Preliminary growth chamber phenotyping experiments showed that introgression 6A segregates as a single recessive resistance gene, and can be scored in growth chamber experiments on a single plant basis. Over 168 F2 individuals and over 300 F2:3 families were phenotyped, and genotyped with SNPs markers in order to narrow down the region of interest (<1.5Mb). Currently, candidate genes are being analyzed. To evaluate fitness and yield, we have developed isohybrid pairs by crossing B73 with/without introgression 6A to several inbred lines (testers). We have found significant differences between the treatments in some pedigrees. Summer yield experiments are being designed to study the influence of the presence or absence of 6A introgression on agronomic traits and disease.

Funding acknowledgement: United States Department of Agriculture (USDA), CGIAR Generation Challenge Program, Corn Growers Assn of North Carolina, NCSU Plant Pathology Dept., Monsanto Outstanding Students Program Fellowship

P192

Fine-Mapping of *Rf4* , a Major Restorer-of-Fertility Gene for c-Type Cytoplasmic Male Sterility in Maize

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C-type cytoplasmic male sterility (CMS) is one of three major CMS types in maize. The inheritance of the fertility restoration is largely unknown and putatively governed by several genes. The *Rf4* gene has been identified to be one of the major restorer genes for c-type CMS. The aim of this study is to fine-map *Rf4* at the upper telomeric end of chromosome 8. Genetic mapping has been carried out in 1317 F_2 individuals derived from a cross between the CMS-parent B37c and the restorer parent K55. Four new PCR-based markers close to *Rf4* were developed by comparative sequencing of the parental lines. *Rf4* mapped 1 cM above the first marker in bin 8.00. All newly developed markers will be available to the public. They will be useful for the marker-assisted selection of c-CMS restorer and maintainer lines.

P193

First steps for breeding galician white corn landraces for traditional-baking bread

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White corn in Galicia (northwest of Spain) was a traditional crop that was grown mainly to obtain flour to elaborate typical galician dishes such as "empanada" or cron bread for home consumption. This crop has survived thanks to small farmers who have continued to cultivate it for years, since the introduction of yellow corn and forage hybrids had shifted completely to the already weak crop of white corn.
The increasing demand for typical products and manufactured following traditional techniques has highlighted the difficulty of the galician bakers for obtaining landraces flour to elaborate traditional corn bread. Therefore, the aim of this work was to evaluate a collection of Galician white corn landraces to select the best of them and breeding them from flour characteristics point of view. The best families obtained by selfpollination from the best landraces evaluated will be mixed to obtain a synthetic variety, combining the main characteristics of these landraces, their adaptation to climatic conditions in the area and better quality flour for traditional bread making than landraces.

Funding acknowledgement: Council for Rural Development- Xunta de Galicia

P194

From QTL Analysis to Evaluation of Near Isogenic Lines for Heterotic QTL in maize.

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Heterosis, or hybrid superiority, has been extensively exploited in maize (*Zea mays* L.), but its genetic basis is not fully understood yet. As a contribution to its study, we undertook a long term research aimed at providing a framework of comprehensive quantitative trait locus (QTL) phenotyping. We first applied a combined QTL analysis following a North Carolina III (NCIII) mating design on genetic materials originated from single cross B73 x H99. For agronomic traits, several heterotic QTL were detected which were characterized by dominant or overdominant gene action, whereas non-allelic interaction proved to be of minor importance. For six of those heterotic QTL, pairs of NILs differing only for alleles at QTL flanking markers were produced, starting from Residual Heterozygous Lines (RHL). The present study was aimed at validating QTL additive and dominance effects for complex traits, as grain yield and its components, and at getting inside into their reactivity to different genetic backgrounds and to mildly stressful environmental conditions. NILs were thus evaluated (*i*) as near isogenic triplets (the two members of a NIL pair and their cross), (*ii*) as crosses with the related inbred lines H99 and B73, and (*iii*) as crosses with unrelated testers. The results showed that: (*i*) QTL effects were in accordance with the effects detected in previous analyses, (*ii*) effects were consistent in different genetic backgrounds and inbreeding level, and (*iii*) heterotic effects varied depending on stress conditions and (*iv*) were more pronounced for complex traits.

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P195

Genetic Analysis and Characterization of Quantitative Resistance to Southern Leaf Blight in Maize

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We describe our progress in mapping and characterizing quantitative trait loci (QTL) for resistance in maize to southern leaf blight (SLB). We have mapped resistance QTL in several populations, including the IBM population and the 5000-line maize Nested Association Mapping (NAM) population. We will present a summary of all these mapping results.

We have fine-mapped QTL on chromosomes 3 and 6 to sub-megabase intervals in which several candidate genes have been identified. These loci confer a ~10% yield advantage under high disease pressure. The expression of two pathogenesis-related genes (PR1 and PR5) during the infection process was up-regulated within five hours of inoculation but we failed to detect consistent differences in gene expression between near-isogenic plants differing for these QTL.

In the NAM population 32 resistance QTL were identified, each of which had relatively small effects on resistance. Additive effects relative to the B73 allele at most QTL varied widely among the 25 parental alleles.. No significant epistatic interactions were detected among the 32 QTL; an additive model of 32 QTL explained 93% of the total genetic variation. Using genome wide association analysis we identified several genes within these QTL with significant associations with disease resistance.

Funding acknowledgement: United States Department of Agriculture (USDA), Pioneer Hi-Bred

P196

Genetic Architecture of Vegetative Phase Transition in Maize

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The transition from juvenile to adult vegetative tissue is a fundamental process that must occur prior to the vegetative to reproductive transition. In maize, the vegetative phase transition from juvenile to adult tissue is under genetic control independent of the vegetative to reproductive transition and the mechanisms controlling this transition are not well characterized. One way to distinguish juvenile vegetative tissue from adult vegetative tissue is the presence of epicuticular wax found on the surface of juvenile leaves. To begin to understand the genetic mechanism underlying vegetative phase change we scored the last leaf with epicuticular wax on 3875 recombinant inbred lines from the Nested Association Mapping population (NAM) as well as 267 intermated B73 x Mo17 recombinant inbred lines (IBM). The last leaf with epicuticular wax varied in the NAM RILs ranged from leaf 5 to leaf 14.25, and ranged from leaf 5.4 to 11 in the IBM RILs. We mapped QTLs within the 25 NAM families as well as the IBMs using composite interval mapping and detected 56 QTL across all NAM populations and 5 QTL in the IBM population. Three major QTL were located on chromosomes 2, 3, and 9. The combined average additive effects of these three alleles equate to almost a 3 leaf difference in transition, or near 40% of the variation observed in the NAM population. The chromosome 9 region contains the gene *Glossy15*, involved in expression of juvenile leaf traits; however, this candidate gene has not been confirmed to be the QTL in this region. The chromosome 2 QTL was detected in 22 of the 25 NAM populations as well as in the IBM population, explaining between 5-55% of the variation in the NAM populations and 16% in the IBM population. These results demonstrate that several major QTL underlie natural variation for this important developmental trait.

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P197

Genetic Control of Matroclinal Haploidy in Maize

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In this presentation, we analyse the inheritance of the ability of producing matroclinal haploids in maize. Genetic analysis was conducted in the system of full diallel crosses according to Hayman. Five lines were included to diallel scheme, ДК276-1, ДК247, ДК293, ДК303/427, ДК205/710, and their reciprocal hybrids. For production of matroclinal haploids every genotype of diallel scheme was pollinated with pollen of marker genotype Zarodyshevij marker krasnodarskij 1 (ZMK-1). "Frequency of matroclinal haploidy" in the diallel set fluctuated from 0,59% to 11,12%. The manifestation of the haploidy was determined by the additive and dominant genetic system. The effects of dominance were mainly concentrated in the same direction. The average degree of dominance for all the loci was incomplete, H1/D < 1. Points of inbreds ДК276-1, ДК247, ДК293 and ДК303/427 were located nearer to the start of the regression line Wr/Vr; they contained mainly dominant alleles (from 75% to 100%), which were responsible for the decrease of "frequency of matroclinal haploidy". For inbred ДК205/710 ratio of dominant and recessive genes reached 25%: 75%. This inbred included the biggest portion of recessive alleles, which determined the increasing of matroclinal haploidy. For "frequency of matroclinal haploidy" incomplete dominance occured (H1/D=0,54), including separate loci. The level of dominance varied in different loci. H1≠H2, so dominant and recessive alleles were spread irregularly among parental inbreds. Positive estimate of F verified the exceeding of the amount or effects of dominant alleles over the recessive ones in this set of lines and hybrids. The high value of heritability in wide sense proved the primary effect of genotypic variance. The significant value of heritability in narrow sense (0,67) confirms the significant role of additive gene effects and permits to make favourable prognosis in phenotype selection for high frequency of matroclinal haploidy. It is very important for production of testers, that are needed for breeding programs of creation of new double-haploid lines.

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P198

Genetic Dissection of Shoot Architecture and Flowering Time Responses to Marginally Differential Photoperiods in Maize

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Understanding the physiological parameters that dictate flowering time response to photoperiod in maize is difficult due to the large number of mechanisms involved. Moreover, the consequences of functional allelic polymorphisms in genes affecting flowering time and related traits are likely dependent on both environmental and genetic context. While it is clear that certain QTL are relevant in nearly all environments, others are relevant only under particular sets of environmental conditions, and teasing apart these QTL-by-environment interactions could help breeders identify the most appropriate QTL targets for their breeding programs. We conducted a two-season study of the IBMRILs grown in Johnston, IA; Marion, IA; and Macomb, IL. Plant height, ear height, and growing degree units till pollen shed and silk extrusion were measured in five trials. From these measurements, 10 additional derived traits were calculated and analyzed, which permitted functional assignment of QTL effects in a phenomic context. QTL searches for all 14 traits were conducted by composite interval mapping on a high density, ~8,000 cM map made with markers connected to the physical map. In all, 15 statistically significant QTL were identified for the primary traits, with four of them showing a strong dependence on the slight photoperiod differences tested in the study. Candidate gene hypotheses constructed using positional and functional results will be presented.

P199

Genetic Diversity of a Maize Association Population with Restricted Phenology

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The developing biofuel industry has increased the need to identify novel variation for biomass quantity and compositional traits for maize and other crops being considered for lignocellulosic ethanol production. Our focus is to study maize as a dual purpose grain and lignocellulosic feedstock crop for the upper Midwest. This focus requires that all genotypes evaluated mature in our environment. For this reason, we have assembled a collection of 645 maize inbred lines that flower no later than 1600 growing degree days (GDD) after planting for association genetic analysis. Phenotypic and genetic diversity was evaluated in this set of inbred lines. Phenotypes scored in 2008 and 2009 include stover yield (tons/ha), morphological traits such as internode length and number, plant height, leaf width, stalk diameter and developmental traits such as flowering time and percentage of leaves with epicuticular wax. Stover yield ranged from 0.13 to 4.37 (tons/ha), internode number ranged from 12 to 25, percentage juvenile leaves ranged from 18 to 71%, and flowering time ranged from 508 to 1562 GDD. Genetic diversity was assessed using a 1536 single nucleotide polymorphism (SNP) Illumina assay. A set of 511 unbiased SNPs were used to determine relationships among inbred lines and to develop population structure and kinship matrices. The polymorphism information content (PIC) for these lines is 0.251 and the gene diversity (expected heterozygosity) is 0.308. Preliminary information on association analysis will be presented, using flowering time as a proof of concept.

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P200

Poster removed

P201

Genetic dissection of multiple disease resistance in maize

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In natural and artificial systems, plants are challenged with numerous pathogen species and races. Since many necrotrophic fungal pathogens share aspects of their pathogenesis strategies, we hypothesized that some quantitative trait loci (QTL) might confer "multiple disease resistance" (MDR). Here we present the results from several genetic approaches we have taken for characterizing MDR and the loci and genes that underlie it.
Syntheses of previous disease resistance QTL studies in rice and maize indicated that disease resistance QTL for different diseases were non-randomly distributed. We observed highly significant genetic correlations for resistance to three fundamentally necrotrophic diseases, southern leaf blight, northern leaf blight and grey leaf spot (SLB, NLB and GLS), in a diverse set of 300 maize lines. Specific allelic variants of a member of the glutathione S-transferase (GST) gene family were associated with resistance to multiple diseases in this population. In various populations including the IBM population, we frequently observed significant correlations between resistances to different diseases. However, most large-effect QTL were disease-specific.

A genetic analysis of SLB, NLB and GLS resistance was performed using the maize Nested Association Mapping (NAM) population. The diverse founder inbred lines showed a strong correlation for resistance to the different diseases. The recombinant inbred progeny also had correlated resistance, though the effect was weaker. Twenty-three genetic positions were identified where quantitative resistance loci for two or more diseases co-localized. To examine the possibility of MDR genes, the estimated allele effects from each founder inbred were compared. At seven loci, positively correlated allele effects provided evidence for MDR genes.
Our analyses so far have suggested that multiple resistance to the three diseases studied here is often due to the accumulation of disease-specific genes but we do have evidence for the existence of pleiotropic genes conditioning MDR.

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P202

Genetic diversity analysis and heterotic grouping of the sweet corn germplasm in China

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With the rapid development of sweet corn industry, a germplasm collection has been established by breeders in China during the last decade. In order to increase use efficiency of these germplasm, we evaluated genetics diversity of 347 inbred lines from the germplasm collection using 57 SSR markers. The average number of alleles per SSR locus was 4.77 with a range firm 2~11. The value of polymorphism information content (PIC) for each SSR locus varied from 0.31~0.734 with an average of 0.489. And then these genotype data was adopted in cluster analysis of the 347 lines to estimate the degree of similarity among these germplasm. Most of accessions (about 95%) were divided into two large groups (Group A and Group B) by UPGMA cluster analysis based on the genetic distance matrix. Group A (182 lines) comprise two major subgroups (66 lines and 99 lines, respectively) and 17 scattered lines. Group B (149 lines) comprise two subgroups (92 lines and 57 lines, respectively). The other accessions (16 lines) have not been classified into the two groups. The cluster results were largely consistent with known pedigree of the lines. So these groups can be regard as heterotic groups. The results were helpful for better understanding genetics background of germplasm and utilization of heterosis in sweet corn breeding.

P203

Genetic of the fitness of the sweet corn mutant *sugary1*

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The survival rate of a defective mutant depends on the carrying genotype, suggesting the existence of genes regulating mutant viability and fitness. Particularly the allele *sugary1* (*su1*) in maize (*Zea mays* L.) can be completely viable or deleterious depending on the genetic background. The main limiting step affecting *su1* frequency is germination, followed by plant development during the heterotrophic phase, the differential performance of *su1* compared to *Su1* decreasing in subsequent stages of plant development. The recombination of crosses between *su1* and *Su1* genotypes reduces the frequency of *su1* phenotypes with the rate of reduction being affected by the genotype of the *su1* and the *Su1* parents. Depending on that variation of *su1* frequency, *Su1* genotypes can be classified as unfavourable for *su1* or neutral. However, when unfavourable *Su1* genotypes are crossed to one *su1* genotype and the cross is self-pollinated, the ration between frequencies *su1*:*Su1* is below the expected 1:3 ratio for some heterozygous ears, while for other ears *su1* grains are even more abundant than expected. The described phenomena are somehow puzzling because the abundance of a defective mutant is contradictory with the expected deleterious effects. We are investigating the possible existence of genes that regulate the viability or fitness through QTL studies using F_2 populations derived from *su1*x*Su1* crosses. The genetic effects and variances affecting *su1* frequency and performance are being estimated through two separate mean generation designs from crosses between unfavourable and neutral *Su1* lines with the same *su1* line. Finally, we are trying to explain the appearance of genetic abnormalities underlying the variability observed (starchy and pseudo starchy grains) when *su1* grains coming from *su1*x*Su1* crosses are successively selfpollinated.

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P204

Genetic regulation of the accumulation of pigments at low temperature

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Maize plants exposed to chilling temperatures undergo chlorophyll breakdown and accumulation of anthocyanins during early development. Although these are general response mechanisms, the severity of these symptoms varies in different genotypes suggesting a regulation mechanism other than just a reduction of biosynthetic activity, but so far all efforts to identify genes related to pigment synthesis as a response to chilling have had a limited success. In order to identify genes involved in the maintenance of chlorophyll and other pigments at low temperatures we followed two different strategies. In a recent evaluation, we observed that the leaves of the inbred line A661 grown at low temperatures are unable to accumulate chlorophyll and accumulate higher levels of anthocyanin than control plants. Chlorophyll and anthocyanin levels were quantified at different temperatures in this inbred line. Likewise, this inbred was crossed to the inbred EP42 and the resulting F2 mapping population genotyped with 72 SSR markers. In a second strategy, we carried out a genetic screening using an EMS-mutated population on B73 background. We evaluated 708 families in a cold chamber and identified 18 putative mutants that were multiplied in the field and retested at low temperatures to confirm the heritability of the mutation. The most promising mutant is unable to produce anthocyanins in the stem at low temperatures. This mutant will be crossed to the inbred Mo17 to obtain the mapping population.

P205

Genetic variability of growth under water deficit in maize: meta-analysis of QTL, modelling and test of allelic effects at consensus QTL

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Expansive growth of leaves or of reproductive organs such as silks are affected by water deficit before any reduction in photosynthesis or root growth. A method combining genetic and ecophysiological modelling has been developed to disentangle the genetic basis of leaf or silk growths response to soil and air water deficits[1]. Analyses revealed a considerable genetic variability for the sensitivities of leaf growth to soil water deficit and to evaporative demand, measured in phenotyping platform. For instance, in collections of recombinant inbred lines or of unrelated lines, some genotypes had their growth stopped at –0.6 MPa while other continued growing until –1.6 Mpa[1,2]. There is therefore a considerable natural genetic variability for sensitivity of growth to water deficit which can be exploited for breeding. Six genomic regions were repeatedly associated across mapping populations with sensitivity to soil water deficit and evaporative demand. They have been tested positively in introgression lines. The dynamic nature of the responses suggests that hydraulic processes significantly contribute to difference in growth maintenance. Comparative mapping in a tropical maize population shows that the genetic determinism of leaf growth maintenance under water deficit is partly common with that of the maintenance of reproductive development[2]. Both modelling and a network of QTL experiments suggest that QTLs for leaf growth translate into differences in yield[3]. These findings may have profound consequences for designing drought tolerant ideotypes.

[1]Sadok W et al (2007) Plant Cell Environment 30, 135–146 ;
[2]Welcker C et al (2007) Journal Experimental Botany 58, 339–349 ;
[3] Chenu K et al (2009) Genetics 183, 1507-1523

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P206

Genotype-by-Environment From a Different Perspective.

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Previous work in our lab (Singh PhD, 2007; Coleman MSc, 2008) using a near-isogenic recombinant inbred line (niRIL) population to identify grain yield QTLs found a profound interaction between the environment and the genotype (GxE) on the expression of grain yield. Their results showed that environments which grouped together (i.e., similar GxE pattern) were primarily from an individual year as opposed to multiple years grouping together and that this year effect was more influential than physical location or plant population densities. The niRIL population gives us a unique tool for studying GxE as that many of the presumed causes of GxE [i.e., differences in: phenology (i.e. flowering time), dry matter accumulation at silking, and leaf area (i.e., light interception)] do not vary in this set of materials. Using 6 years (2004 – 2009) of weather data and 41 trials of grain yield data from the niRIL, we are beginning to dissect the E component of the GxE interaction. We are initially focusing on air temperature and soil moisture because they are the most crucial weather parameters in corn development (Wiatrak, 2009) and as a result the timing and amounts of these parameters can negatively influence crop development and thus reduce grain yield. Using hourly air temperature and precipitation values, and soil type information, we will integrate these parameters into a "soil moisture budget" which allows us to compare available soil moisture throughout the developmental stages and the grain-filling period. Information gained through this approach should help us to better understand: (1) when during the life-cycle are the most sensitive times for the environment to influence grain yield expression, and (2) what is the magnitude or threshold of the changes in air temperature and precipitation that result in GxE effects.

P207

High-resolution mapping and genotyping of maize using long oligonucleotide microarrays

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The completion of the maize genome sequence [1] has facilitated the development of high-resolution microarray based genomics tools. Recent exploration of genomic complexity between two maize inbreds has revealed unparalleled structural variation among strains within the same eukaryotic species [2]. Many of the differences observed between inbreds were found to be presence-absence-variation (PAV). Sequences subject to PAV between the B73 and Mo17 inbreds are found throughout the genome. A large proportion of the sequence missing from Mo17 is single copy and likely genic in origin [2]. The existence of sequences present in one-haplotype but entirely missing in another suggested that such sequences may be useful as dominant molecular markers for high-resolution mapping and genotyping by comparative genomic hybridization (CGH). From a list of over 1.9 million candidates, 200,000 single feature polymorphisms were identified via a B73 x Mo17 comparison and used to genotype two IBM recombinant inbred lines (RIL). Several analytical paradigms for genotyping were explored and the results were assessed by a comparison to >10,000 known marker genotypes for these RILs [3]. The use of multiple hybridization replicates provided ~97% accuracy while a single genotyping reaction provided ~95% accuracy on a per marker basis. Single hybridization genotyping accuracy could be increased to >99% by utilizing information from adjacent probes (i.e. BAC level resolution), while still allowing recombination break points to be determined within approximately 110Kb resolution. A similar approach was used to perform fine mapping for the breakpoints in a translocation (T5-6b). The break-points for T5-6b were mapped to an ~80Kb interval on both Chr5 and Chr6 by performing CGH with the DpDf segmental aneuploid off-spring.

1: Schnable et al., 2009, Science
2: Springer et al., 2009, PLoS Genetics
3: Liu et al., 2009, PLoS Genetics.

P208

How many SSR and SNP markers for population structure and genetic diversity analyses in a commercial maize breeding program?

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Information about the genetic diversity and population structure in elite breeding material is of fundamental importance for the improvement of crops. The objectives of our study were to (i) examine the population structure and the genetic diversity in elite maize germplasm based on simple sequence repeat (SSR) markers, (ii) compare these results with those obtained from single nucleotide polymorphism (SNP) markers, and (iii) compare the coancestry coefficient calculated from pedigree records with genetic distance estimates calculated from SSR and SNP markers. Our study was based on 1537 elite maize inbred lines genotyped with 359 SSR and 8244 SNP markers. The average number of alleles per locus, of group specific alleles, and the gene diversity (D) were higher for SSRs than for SNPs. Modified Roger's distance (MRD) estimates and membership probabilities of the STRUCTURE matrices were higher for SSR than for SNP markers but the germplasm organization in four heterotic pools was consistent with STRUCTURE results based on SSRs and SNPs. MRD estimates calculated for the two marker systems were highly correlated (0.87). Our results suggested that the same conclusions regarding the structure and the diversity of heterotic pools can be drawn from both markers types. Furthermore, although our results suggested that the ratio of the number of SSRs and SNPs required to obtain MRD or D estimates with similar precision is not constant across the various precision levels, we propose that between 7 to 11 times more SNPs than SSRs should be used for analyzing population structure and genetic diversity.

P209

Identification of QTL for yield-related traits induced by space flight in sweet corn

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Space flight is an effective approach for induced mutation in plant seed. Some seeds of sweet corn inbred line 1132 were flown on a recoverable satellite for 18 days in order to acquire some favorable mutation for genetics breeding. After returning to earth, the seeds were germinated and grown. Significant changes for some important trait, such as increase in ear diameter, ear length, row number, ear weight and kernel weight per ear, were observed in plants developed from these seeds. Subsequently, an inbred line 751 was developed from the mutated plant. Simple sequence repeat (SSR) markers which were polymorphic between 1132 and 751 were screened among about 500 SSR markers covering the entire maize genome. It has been shown that only SSR markers on chromosome 9 have the high frequency polymorphism. Then, linkage map consisting of the 28 marker loci on chromosome 9 was constructed using the genotype data identified in 342 $F_{2:3}$ lines derived from a cross between 1132 (wild type) and 751. And above-mentioned traits were evaluated in the $F_{2:3}$ population. The phenotype data, in combination with the linkage map, were used to conduct composite interval mapping to locate QTL for above traits. Six QTLs were identified in three QTL regions (9.01, 9.03, 9.06). For ear diameter, a major QTL (9.03, near umc1634) at a very high LOD score of 12, explaining 16% of the phenotypic variation, and two minor QTL (9.01, 9.06) were detected. In addition, two QTLs, for row number and kernel weight per ear respectively, located on 9.03 and partial overlapped with the major QTL for ear diameter. A QTL for ear length, accounting for 4.2% of the variation, was located on chromosome 9.06, overlapped with the QTL region for ear diameter. No QTL was detected for ear weight under the significant threshold LOD scores. These results indicated that some favorable mutant alleles for increase yield had induced in space environment. Fine mapping for these QTLs will be implemented aimed at mining these alleles and promoting high-yield breeding in sweet corn.

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P210

Influence of maternal parentage and season on the in vivo haploid induction rate in tropical maize

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The haploid induction rate (HIR) is of crucial importance for in-vivo production of double haploid (DH) lines in maize (Zea mays L.). It depends strongly on the inducer used as a paternal parent and is also influenced by the maternal parent and the environmental conditions. We used tropical maize germplasm as a maternal parent and studied (i) the genotypic variance of HIR among maternal parents, (ii) the influence of summer and winter seasons, and (iii) the relative contribution of general combining ability, specific combining ability, and genotype × season interactions. Ten tropical inbred lines were mated in a half diallel design. The resulting 45 F1 crosses were pollinated with the inducer hybrid RWS × UH400. HIR was evaluated during the summer (wet) 2008 and winter (dry) 2009 seasons at Agua Fria, Veracruz, Mexico, and ranged from 1.4 – 14.5% with an average of 6.8%. There were highly significant ($P < 0.01$) differences between two seasons, HIR being higher during the winter (%) than the summer season (%). Genotypic differences among the 45 F1 crosses were highly significant ($P < 0.01$) and mostly explained by GCA effects, whereas genotype × season interactions were comparatively small. Our study underpins the importance of combining the right maternal genetic background and growing conditions to enhance the efficiency of production of DH in tropical maize in the initial stages.

P211

Integrating Pre-Breeding with Cultivar Development in Maize

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The NDSU maize breeding program is the most northern maize breeding program in North America and its goals are to adapt exotic elite genetically broad-based germplasm, improved adapted germplasm, develop unique inbred lines, populations, and NDSU x industry hybrids, and educate/train applied plant breeders. Over 100 experiments (mostly based on incomplete block designs) each ranging from 50 to 256 genotypes in over 50,000 plots were conducted across 42 environments in 2008 and 2009. Over 35,000 total nursery rows were managed with 16 acres of summer pollinating nursery just in 2009 with over 100,000 pollinations. NDSU has continued the only program developing early maturing drought-tolerant products with drought managed sites since 2001 as the western ND market is not large enough for industry investment. We have moved elite tropical and late temperate maize northward with minimal investment. Unlike expensive genomic efforts on isolating early-maturing genes and QTL for potential MAS, we have made significant genetic progress at a rate of 2 to 3 days earlier per year by screening 200,000 plants across populations with a very simple approach at less than one penny ($0.01) of screening cost per plant. We have worked with unique tropical and early maturing alleles to increase the genetic diversity of U.S. northern corn hybrids, not present in the B73 genome just sequenced. Winter nurseries allowing more than two seasons per year have helped speed up the development of unique exotic <90RM NDSU EarlyGEM lines. These have shown not only to be 12 to 20 days earlier than original versions, but also greater grain yield (10.4 vs. 9.2 t/ha), test weight (72.5 vs. 70.1 kg/hL), extractable starch (67.8 vs. 64.2 %), fermentable starch (16.6 vs. 16.4 %), grain oil (4.3 vs. 3.5 %), grain protein (10.5 vs. 9.4 %), and up to 194 % greater yield under intense drought conditions when compared to top industry hybrids at similar grain moisture at harvest. There has, during the past 10 years, been a significant erosion of plant breeders at universities to educate the next generation of breeders. The demand and interest of students to be trained in applied corn breeding at NDSU has significantly increased.

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P212

Introgression from hybrids varieties into landraces populations of maize (*Zea mays* ssp. *mays* L.)

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Landraces are domesticated local plant varieties that did not experience a deliberate and intensive selection during a formal breeding program. They are subjected to the combined actions of natural and human factors which determine the agro-ecosystem. Thus landraces represent a very important source of genetic diversity to exploit for conservation, plant breeding, and for evolutionary studies.
In Italy flint maize landraces are still cultivated in order to produce traditional food, for which the dent corn is not suitable from a quality point of view. Here, using 21 SSR and 168 AFLP molecular markers, we compared two landrace flint maize collections from Central Italy obtained in two different periods, spanning 50 years: an 'old' collection undertaken during the 1950s, thus before the introduction and spread of hybrid varieties and a 'recent' collection (2000-2005). For comparison a sample of improved germplasm including hybrids and inbred lines was also used. The population structure and divergence analysis showed that the introduction of hybrid varieties led to a significant amount of introgression from hybrid varieties into the recent landrace collection. Selection tests were performed in order to disentangle the effects of migration and selection in determining the introgression seen. Overall the effect of selection was small and on average favoured the introgression from modern maize into landraces. Interestingly the outlier loci identified suggested a selection both acting between the flint and dent gene pools, and for changing environments or in favour of new alleles introduced by migration from hybrids over the last 50 years. Overall, these results show the potential of landraces to be exploited as models for studies aimed at the detection of loci that control important adaptive variants and agronomic traits.

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P213

Joint linkage-linkage disequilibrium mapping increases QTL detection power: a case study of anthesis-silking interval and grain yield for improving drought tolerance in maize

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The power of quantitative trait locus (QTL) mapping in plants has been limited by population size, marker allele diversity and the inherent properties of specific mapping methodologies. Several of these rate limiting factors have been addressed in this study by joint linkage-linkage disequilibrium (LD) mapping using 2052 single nucleotide polymorphism (SNP) markers and three recombinant inbred line (RIL) populations, 105 introgression lines (ILs), and 200 inbred lines collected from global maize breeding programs. The joint linkage-LD mapping has been achieved in two ways: (1) parallel linkage and LD mapping and (2) integrated linkage-LD mapping, by using biparental and inbred-line populations independently and combined, respectively. LD mapping was implemented using single SNPs and their haplotypes. Two important traits for maize drought tolerance, anthesis-silking interval (ASI) and grain yield (GY), were measured under both well watered and water stressed conditions. Analysis of population structure clearly separated the biparental populations from the inbred lines. Compared to using single SNP data, the use of haplotypes increased the polymorphism information content from 0.253 to 0.434 and improved mapping power with the sum of explained phenotypic variation (EPV) increasing from 7.5 to 21.3%. As population sizes increased from 200 (inbreds) to 522 (inbreds, ILs and RILs), power in single SNP-based LD mapping significantly increased with P-values decreasing from 2.88×10^{-4} to 8.71×10^{-6} and the sum of EPV increasing from 8.3 to 21.3%. The integrated linkage-LD mapping significantly improved the mapping power, identifying four marker-trait associations that were not detected by either method alone. A total of seven major QTL clusters for ASI and GY were identified across six chromosomes. Four specific maize genes associated with drought tolerance have been confirmed by different mapping approaches, including CYP71C1 gene for cytochrome P-450, salt-inducible putative protein serine/threonine/tyrosine kinase, receptor expression-enhancing protein 3 (SPG3) and AGL2 MADS box family (MADS 27).

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P214

Lessons From *Dwarf8* on the Strengths and Weaknesses of Structured Association Mapping

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Association mapping has it strengths in resolution and allelic richness but is potentially confounded by historical relationships and selection patterns. Here we reanalyze one of the first generation of structured association (SA) mapping studies of the *Dwarf8* locus and its association with flowering time in maize with the full range of new mapping populations, statistical approaches, and haplotype maps. For this strongly confounded trait, we found that basic SA methods do a poor job in estimating potential phenotypic effect in the region, while mixed model approaches perform substantially better. Combined analysis with maize NAM (a multi-family crossing design) suggests that most if not all of the QTL effects at the general location of *Dwarf8* are from rare extended haplotypes that extend to other linked QTL. Population bottleneck, selection patterns and haplotype structure observed in the region suggest that the *Dwarf8* locus may be confounded with the structure of the population and that selection of multiple traits has influenced the region. Overall, this reevaluation provides insights into how modern association- and linkage mapping with hapmap tools can make for more robust results going forward.

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P215

Maternal control of interploidy lethality

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Crosses between diploid and tetraploid maize result in aberrant seed development and failed seeds. When the genomic excess is inherited through the pollen (diploid x tetraploid crosses), cells fail to cellularize, storage products are aberrantly regulated and endoreduplication, repeated rounds of nuclear genome replication without division, is delayed. This regulation of endosperm cell cycles, development and deposition of storage materials is sensitive to both gene dosage and parent-of-origin effects. Classical experiments in maize, and more recent molecular genetic experiments in Arabidopsis, indicate that some of these effects are contributed by the epigenetic regulation of gene expression in either the endosperm itself, or the preceding gametophyte generation. Previously we demonstrated maternal genetic control of endoreduplication in maize endosperm. We are now taking a QTL approach to dissect the genetic architecture of interploidy lethality, and cell cycle perturbation, using germplasm with demonstrated effects on the cell cycle. Lines with high survival and low survival as maternal parents in mismatched-dosage crosses were hybridized and the segregating embryo sacs on the F1 were fertilized by pollen from a tetraploid parent. As the vast majority of seeds fail, surviving progeny exhibit transmission distortion at molecular markers linked to QTL affecting the interploidy barrier in maize. We have taken a similar approach in Arabidopsis uncovering maternal sporophytic controls, the TTG2 transcription factor, as well as segregating post-meiotically expressed QTL that induce distortion at linked markers in similar diploid F1 x tetraploid crosses. Using TTG2 as a starting point we have identified a genetic network that controls interploidy lethality in Arabidopsis and may indicate a more general coordination of maternal and offspring development shared with maize.

P216

Meta-Analysis of QTL for Traits involved in Silage Quality of Maize and Comparison with the Position of Candidate Genes

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A meta-analysis of QTLs associated to plant digestibility and cell wall composition in maize was carried out using results from 11 different mapping experiments. Statistical methods implemented in “MetaQTL” software were used to build a consensus map, project QTL positions and perform meta-analysis.

Fifty-nine QTLs for traits associated to digestibility and 150 QTLs for traits associated to cell wall composition were included in the analysis. We identified 26 and 42 metaQTLs for digestibility and cell wall composition traits respectively. Fifteen metaQTLs with confidence interval (CI) smaller than 10cM were identified. As expected from trait correlations, 42% of metaQTLs for digestibility displayed overlapping CIs with metaQTLs for cell wall composition traits. Coincidences were particularly strong on chromosomes 1 and 3.

In a second step, 356 genes selected from the MAIZEWALL database as candidates for the cell wall biosynthesis pathway were mapped onto our consensus map. Co-localizations between candidate genes and metaQTL positions appeared globally significant based on chi-square tests.

This study contributed to identify key chromosomic regions involved in silage quality and potentially associated genes for most of these regions. These regions will soon be compared to QTLs found in a multiparental experimental design of 1555 double haploid individuals issued from 18 connected populations derived from 13 parental lines.

P217

Meta-analysis of QTLs controlling root length of maize

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Traits related to the root length of maize (*Zea mays* L.), reported by 15 QTL studies of nine mapping populations, were subjected to a QTL meta-analysis. Included were QTLs for elongation rate, root number and weight at the seedling stage as well as proxy measures for root length in the field, such as root pulling force and root capacitance. Traits were grouped according to ontology, and a system of abbreviations is proposed to unambiguously identify the different root types and branching orders. The nine maps were merged into a single consensus map, and the number of putative QTL clusters (MQTLs) per chromosome was determined using the software MetaQTL. A total of 161 QTLs was grouped into 24 MQTLs and 16 individual QTLs. For example, the MQTLs located in bins 1.07 and 3.06 combined 11 and 8 QTLs, respectively, and were detected in more than three populations. We did not detect a consistent pattern of additive effects across developmental stages or among root types. This indicates a considerable number of linked loci at the MQTL positions but may also indicate effects of QTL-by-environment interactions. Seven MQTLs harbored root traits, which had been reported to be co-located with QTLs for grain yield or drought tolerance in the field. The most consistent collocations among them were those for the number and weight of the seminal roots. At least six loci (located in bins 1.07, 2.04, 2.08, 3.06, 6.05 and 7.04) are worthy candidates for further evaluation. In conclusion, we provide a comprehensive overview of QTLs affecting root length of maize and discuss the question how to shape efficient root systems in maize.

P218

Modeling Stalk and Root Lodging as a Zero-Inflated Process

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Zero-inflated data (ZID) arise when observations are generated from a mixture of some known statistical distribution and a null distribution. In biological data, this occurs when conditions needed for the expression of a phenotype are not met for all observations. Stalk and Root lodging (RL and SL) are two important factors in the commercial viability of maize hybrids and are typically measured as counts of the number of lodged plants in a given plot. The phenotypic distributions of these traits show a high proportion of zeros, suggesting the traits may follow a zero-inflated Poisson distribution (ZIP). To test this theory, a Bayesian ZIP model was applied to several SL and RL datasets. The 95% high density posterior intervals showed significant mixing proportions of the null and Poisson distributions, indicating SL and RL do follow a zero-inflated process. To evaluate the impact of modeling assumptions on the predictions of genetic merit when data are distributed as ZIP, a simulation study was conducted. Data were simulated using both Poisson (D1) and ZIP (D2) distributions, and analyzed using models assuming Poisson (M1) and ZIP (M2) distributions. For D1 there was no difference in the predictive accuracy of M1 and M2; however, M2 showed significantly more accurate predictions of genetic merit for D2, suggesting zero-inflated models may improve accuracy of SL and RL analysis.

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Multidimensional analysis of maize root system architecture in vivo.

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The health and productivity of a plant depends on its ability to recognize and adapt to its environment. Environmental cues are therefore integrated with endogenous developmental programs to control the spatial and temporal growth of both shoots and roots. A comprehensive understanding of root system architecture (RSA) has been hampered by severe limitations in available observation methods. Recently, a robust gel-based technique was developed that allows three dimensional observations of root systems in vivo. The resulting high-quality images were used to phenotype both standard and novel RSA traits for rice roots. We applied this technique to several maize inbred lines, revealing clear variation among their root system topologies. Furthermore, we used sets of images for each plant at to computationally reconstruct three dimensional models of the root systems at various time points. Collectively, these data represent the first steps to a comprehensive understanding of maize root system dynamics. We are using this method to analyze root development in each of the nested association mapping (NAM) parental lines, with the eventual goal of identifying and fine-mapping QTLs responsible for RSA traits. Furthermore, our approach can easily be used to study root system dynamics under a range of environmental conditions, which will allow us to study the integration of genetics and the environment.

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P220

Mutant-assisted exploration of natural variation underlying R gene-mediated immunity in maize

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The hypersensitive response (HR) is arguably the most important defense response in plants, but details of how it is controlled and executed remain patchy. We used a novel genetic technique called MAGIC (Mutant-Assisted Gene Identification and Characterization) to identify an HR-modulating locus in maize. MAGIC facilitates identification of naturally occurring alleles underlying phenotypic variation from diverse germplasm using a mutant phenotype as a "reporter". In this case the reporter phenotype is caused by a partially-dominant autoactive disease resistance-gene Rp1-D21 which causes HR lesions to form spontaneously. Genetic background profoundly affected the Rp1-D21 phenotype. B73 and Mo17 partially suppressed and enhanced the Rp1-D21 phenotype, respectively. By crossing the Rp1-D21 gene into the IBM population we were able to map and identify Hrml1 (HR-modulating locus 1), a locus responsible for modulating the Rp1-D21 phenotype, on chromosome 10. Loci with smaller effects were identified on chromosomes 1 and 9.
We are now using the NAM and maize association-mapping populations with MAGIC to uncover additional Hrml loci and to clone the underlying genes. We are also using "pseudo-F2" populations to identify recessive Hrml loci.
Furthermore, we are using computational image analysis to characterize the phenotypic expression of Rp1-D21 in diverse germplasm in different environments. Results from these analyses will be presented

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P221

Native Resistance to Corn Rootworm Beetles: The Path from Germplasm Screening to Allele Deployment

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The western corn rootworm beetle (WCRWB), Diabrotica virgifera virgifera LeConte, is one of the most damaging pests of maize and results in significant economic losses due to diminished yield and expensive control measures. Much of the research related to this problem has focused on identifying resistant germplasm without addressing the underlying mechanisms. Our goals are to identify new sources of resistance and to genetically and mechanistically characterize their effects. Here we report on the screening of GEM germplasm to identify new sources of native resistance to larval feeding by CRW beetles, breeding efforts to generate segregating F2 and BC1 analysis populations, and the use of these populations for identification of alleles that confer increased resistance or tolerance. Parental lines representing both stiff-stalk and non-stiff stalk heterotic groups were selected after repeated evaluation in a high-feeding-pressure trap nursery and used to generate segregating populations. Extensive variation in root injury and lodging was seen in the stiff-stalk heterotic group, offering an opportunity to map and genetically characterize alleles that reduce larval feeding damage. Conversely, the non-stiff stalk heterotic group showed the greatest variation in adult beetle feeding on above ground plant organs. Preliminary QTL mapping of root injury within the stiff stalk material was conducted using a set of 104 SNP markers across 264 BC1 individuals and 120 F2 individuals. Several positionally coincident QTLs of moderate effect were identified in both the F2 and BC1 populations. These findings demonstrate that allelic differences in the host confer varying levels of native resistance, and further, that these functional polymorphisms are experimentally tractable in field experiments. We plan to use the heritable differences we have characterized to generate nearly isogenic lines for use in mechanistic investigations.

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P222

Nested Association Mapping for Maize Tassel and Ear Architecture

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Maize inflorescence structures, the tassel and ear, provide researchers with the opportunity to better determine genetic components of the development of complex architectures. QTL mapping analysis utilized the Nested Association Mapping (NAM) population. Tassel phenotypes were collected from multiple replications, these traits plus calculated derived traits were used in the QTL analysis. Significant QTL were found in regions described in other studies, as well as new regions not previously implicated for tassel inflorescence architecture. For tassel inflorescence architecture, number of QTL described per trait ranged from 8 (ratio of central spike length to central spike spikelet density, r^2=11%) to 24 (tassel branch number, r^2=42%). Candidate genes for QTL detected in the NAM study include ramosa1, sparse inflorescence1, barren stalk1, tasselseed4, and other genes. For some of the candidate genes nearly every phenotypic trait showed an associated QTL in the region. The integration of NAM with association analysis is useful for candidate gene discovery and validation. As an example, ts4 is possibly mapping to a large QTL cluster in NAM, and potential significance in association analysis was found, both potentially linking it to variation in tassel architecture traits. Full results on tassel and ear architecture QTL will be presented.

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P223

New inducers of maternal haploids in maize

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For further extending the technology of in vivo haploid induction in maize breeding and research, there are new requirements for maternal-haploid inducers. Besides a high rate of haploid induction, new inducers have to possess a reliable system of identification marker genes and improved plant traits.

New inducers, PHI (Procera Haploid Inducers), have been derived from a hybrid between two inducer lines - MHI (Chalyk, 1999) and Stock 6 (Maize Genetics Cooperation Stock Center). The PHI inducers displayed haploid induction rates of 10 to 17%, almost twice higher in comparison with the best initial inducer – MHI (7%). The new inducers are the carriers of the R1-nj, B1 and Pl1 marker genes allowing haploids to be indentified among dry kernels, seedlings and mature plants. Plant height in most of the PHI inducer lines was about 2 meters; they had large tassels with good pollen production and a rather good seed set after their self-pollination.

P224

Mapping and Cloning of a major QTL against head smut in maize

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Head smut, caused by the host-specific fungus Sphacelotheca reiliana (Kühn) Clint, is a soil-borne and systemic disease in maize. Head smut cause heavy economic damage in Southern Europe, North American and Asia. In 2002, maize yield lost amounted to 10-15% in Northern China due to epidemic head smut.

We previously initiated QTL mapping of maize resistance to head smut using a backcross population of the cross of Ji1037 (highly resistant) and Huangzao4 (highly susceptible), and identified a major QTL located on bin2.09 that accounts for 36% of the total phenotypic variation. Fine-mapping of the major QTL was conducted in multiple backcross generations. In each backcross generation, recombinants were screened with the markers developed in the QTL region and then backcrossed to the recurrent parent Huangzao4. The resistance of a recombinant to head smut is obtained based on investigation of its progeny. If the progeny with the donor region are more resistant than those without the donor region, this indicates the presence of resistance in the parental recombinant. Analysis of both resistance and donor region for every recombinant allowed delimitation of the resistance QTL. Finally, the major QTL was delimited between markers STS6 and STS7.

BAC clones covering the major QTL region were screened and sequenced. Within the STS6 /STS7 interval, Ji1037 has the longest segment of 170kb; while, Huangzao4 only remains 56kb with a big deletion. We compared to B73 genome sequence and also found a deletion in this region. Three putative genes in the QTL region are probably associated with resistance to head smut. Validation of the candidate resistance genes is in process via transformation of three kinds of vectors, complementary, RNAi interference and over-expression vectors.

Marker-assisted-selection has launched to improve 10 elite but susceptible maize inbred lines in China and, on average; an increase of 30% resistant percentage was obtained.

P225

Phenotypic analysis of biomass and moisture contents in testcrossed double-haploids of maize

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Physiological studies indicate that biomass accumulation in the maize kernel begins after a lag phase and continues until the kernel reaches physiological maturity. These same studies suggest that accumulation is not linear but rather follows a sigmoid curve over time. Studies of moisture content suggest a quadratic relationship with time across all phases of kernel growth and maturation. While the respective canonical shapes of these curves are expected to be sigmoid and parabolic, there are not canonical models associated with moisture accumulation and loss or biomass accumulation. The intense evaluation of the biomass accumulation and moisture accumulation and loss will be used to determine an accurate model to describe these curves and will also provide novel opportunities to identify appropriate phenotypic measures for use in QTL mapping. Repeatable differences in the shapes of the curves among doubled haploid testcrosses will provide evidence of broad sense heritability and potential for genetic mapping.

This study takes place over two years. In 2009, biomass and moisture contents throughout kernel growth were measured in a sample of 65 segregating double-haploid lines that were derived from a cross between two lines and then testcrossed to an elite line for evaluation as hybrids, and five check hybrids provided in order to determine the repeatability (broad sense heritability) of the characteristics of the biomass and moisture content curves. Mapping the phenotypic variation will be attempted in 2010 using appropriate testcross families. Biomass and moisture contents for the five check hybrids will be presented along with estimates of repeatability from the 2009 study.

P226

Progress towards the Positional Cloning of Three Northern Leaf Blight Quantitative Disease Resistance Loci

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To elucidate the mechanisms of quantitative disease resistance, we are examining quantitative trait loci (QTL) for resistance to northern corn leaf blight (NLB), an important disease of maize. A map-based cloning approach is being used to uncover genes associated with three previously identified NLB QTL at maize bins 1.02, 1.06 and 8.06 (designated *qNLB1.02*, *qNLB1.06*, and *qNLB8.06*). Each of these three loci exhibits unique resistance characteristics. *qNLB1.06* and *qNLB1.02* were found to be effective mainly against fungal penetration and colonization, respectively. In addition to NLB, *qNLB1.06* confers resistance to Stewart's wilt, and *qNLB1.02* conditions resistance to Stewart's wilt and common rust. *qNLB8.06*, on the other hand, conditions race-specific NLB resistance. An allelism test showed that *qNLB8.06* is identical, allelic, or closely linked and functionally related to a major gene locus *Ht2*. The three loci are currently at various stages of the fine mapping process. At present, *qNLB1.02* comprises a region of 17.9 Mb, *qNLB1.06* spans a region of 15.3 Mb, and *qNLB8.06* has been narrowed to 0.46 Mb. Loci are being further refined using breakpoint analysis and association analysis, including nesting association mapping. For *qNLB1.02* and *qNLB1.06*, 1239 and 815 recombinants were identified, respectively, in the summer of 2009. By evaluating these recombinants in 2010, positions will be significantly narrowed. For *qNLB8.06*, out of 12 annotated genes in the fine-mapped region, three candidate genes including two encoding protein kinases and one encoding a protein phosphatase were identified.

P227

QTL detection for grain weight and grain filling rate at four stages after pollination and three starch-related enzymes at 30 DAP

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Grain weight is one of the three direct yield components, and developed through a long period of grain filling in maize. Its genetic basis needs to be extensively revealed through all developmental stages. In this study, a total of 258 F9 RILs, derived from a cross between a dent corn inbred Dan232 (with large grain) and a popcorn inbred N04 (with small grain), were evaluated for 100 grain weight (100GW) at 10, 20, 30, 40 days after pollination (DAP) and the activity for three starch-related enzymes (AGPP, GBSS, SSS). The grain filling rate (GFR) was calculated during six stages. The variance components of genotype and genotype × environment interactions for all traits, and those of environment for most traits, were significant. The heritability for all traits was high (from 0.765 to 0.970) except GFR at 30-40 DAP (0.407). The final 100GW (40 DAP) was significantly correlated with all other traits. QTL mapping were conducted for all traits under each environment and in combined analysis using composite interval mapping (CIM) and multiple interval mapping (MIM). Except that no QTL were detected for GBSS, 1-4 QTL were detected each for other traits. QTL at bin 7.02-7.03 in the same marker interval umc2057- umc1567 was consistently found for most traits. In our previous studies QTL for 100GW in this RILs population and its original F2:3 generation, and four EST cloned from 20 DAP endosperm for the same two parents have been detected/located at the same marker interval. Also, some genes and ESTs with related functions, and QTL for grain weight detected in other researches have been located at bin 7.02-7.03. Obviously, our results do provide useful information for identifying and cloning related genes for grain weight. It is worth to concentrate further researches on this marker interval.

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P228

QTL mapping for Pericarp Thickness in sweet corn

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Pericarp thickness is of great importance to the tenderness and sensory of sweet corn. Thin pericarp is a major selection criterion in quality breeding of sweet corn. To better understand genetics mechanism of pericarp thickness and mine favorable alleles, we detected quantitative trait loci (QTL) for the pericarp thickness using a population comprising 190 BC_1F_2 families derived from the cross of Richao-1 (thin pericarp, 56.57μm) ×1021 (thick pericarp, 100.23μm). Pericarp thickness of the parents and families was measured by micrometer. A linkage map of 102 simple sequence repeat (SSR) markers was constructed. Composite interval mapping was conducted using the phenotype data and the linkage map to locate QTL. Five QTLs were identified for pericarp thickness on chromosome 2, 3, 5, 6 and 8, comprising an additive QTL and two pairs epistatic QTLs. Among these QTLs, only the QTL located on 8.04 showed additive effect, explaining 8.2% of the phenotypic variation. Additive × additive epistatic effects for pericarp thickness were showed between QTL in 2.01 and QTL in 6.05, with estimated 6.6% of the phenotypic variation. Similarly, the other interacting QTL pair was located on 5.06/6.01 and explained 12.5% of the phenotypic variation. The results suggested that epistasis, as well as additive effect, played an important role in the genetic basis of pericarp thickness.

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P229

QTL mapping for root characteristics at the seedling stage in maize

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In order to elucidate the genetic control of root architecture in maize, a collection of maize introgression library (IL) lines developed from the cross between two parents contrasting for root traits (B73 and Gaspé Flint) was studied. The IL collection includes 75 lines, most of which retain one single chromosome introgression of the donor genome (Gaspé Flint) of an average length of ca. 40 cM. It has been estimated that ca. 70% of the Gaspé Flint genome is represented within the collection. The IL lines were evaluated for root characteristics by applying two different methodologies, i.e. a paper-roll based protocol and a pot-growing system (seedlings grown until the fourth-leaf stage in sand/clay pebble pots). Particularly striking differences were observed between the two parental lines and among the IL lines for the number of seminal roots developing from the scutellar node. B73 produced an average of 2.8 seminal roots per plant while Gaspé Flint did not develop any seminal root. Among the IL lines, a few showed a Gaspé-like phenotype for seminal root number, implying that the QTLs controlling this trait are localized on the corresponding introgressions. A major QTL for number of seminal roots (*Seminal root 1, Sr1*) was localized on chromosome 1S, ca. 10-15 cM away from the root architecture locus *Rtcs1* (Taramino et al., 2007, Plant J. 50:649-659). Phenotypic expression and fine genetic mapping data seem to indicate that the two loci do not coincide. Positional cloning of *Sr1* is underway.

P230

QTLs and genes for Mediterranean corn borer resistance

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Ostrinia nubilalis (ECB) and *Sesamia nonagrioides* (MCB) cause important losses in temperate maize production as a consequence of stalk tunneling, but QTL analyses for resistance to stalk tunneling were mostly restricted to ECB. RIL populations derived from B73 × Mo17 and EP42 × EP39 were used to detect QTLs for resistance to stem tunneling by MCB. We detected two QTL at bins 1.06 and 9.04 with additive effects of approximately 4 cm of tunnel length in the B73 × Mo17 RIL population, and those QTLs were close to QTLs found previously for ECB resistance. Three QTLs were detected for stalk tunnel length at bins 1.02, 3.05 and 8.05 in the EP42 × EP39 RIL population. The QTLs at bin 3.05 and 8.05 were co-located to a QTL for plant height and grain humidity, and to a QTL for yield, respectively. Now, we will try to identify the resistance gene behind the QTL at bin 9.04 because the same region has been consistently associated to corn borer resistance in other populations. We have chosen one candidate gene, GRMZM2G116452, which encodes a peroxidase precursor. The possible relationship between polymorphisms at this gene and resistance to corn borers will be studied using a panel of 282 inbred lines which represent most of the genetic diversity of public inbred lines. The seed of the inbred lines and the genotypic data are available within the PANZEA project (http://www.panzea.org/lit/germplasma.html). On the other hand, we propose to do fine mapping with near-isogenic lines in the region 8.05 in which we have detected a QTL for resistance and yield. The objective will be to know if there is a QTL with pleiotropic effects on both characters or the effects are due to two linked QTLs.

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P231

QTLs controling the deep-seeding tolerance in maize

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Deep-seeding tolerant seeds can emerge from deep soil where the moisture is suitable for seed germination, breeding deep-seeding tolerant cultivars is becoming increasingly important in semi-arid regions. We found that deep-seeding tolerance was caused in great part by mesocotyl elongation, which was controlled by several loci according to previous research (Troyer, 1997). To further dissect the QTL (quantitative trait locus) controlling deep-seeding tolerance related traits, we selected a tolerant maize inbred line 3681-4 and crossed it with an elite inbred line-X178, forming an F2 population and its derivative F2:3 lines. The molecular linkage map composed of 173 molecular markers was combined with the phenotype data for emergence rate (ER), vigor index (VI) and mesocotyl length (ML), 15 QTLs were detected in all, including 8 for ML, 4 for ER, 3 for VI. The coincidence of QTL for the three traits was ubiquitous. We got one BC3F2 population by selfing a BC3F1 plant which was heterozygous at the markers around the major QTL (qML-10), we found this QTL explained more phenotypic variance in this population than that in F2 population. This study provides the opportunity for improving the deep-seeding tolerance of X178 and further for breeding deep-seeding tolerant cultivars.

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P232

QTLs for Yield Components in Maize: complexity reduction by Long-Range PCR and preliminary results of association mapping analysis.

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We have launched a pilot scheme aiming at cloning a QTL for Yield components in maize. We targeted gene-rich regions for dense SNP discovery in order to perform accurate association studies using low LD panels. Thus, we could avoid the cumbersome development of recombinants that are required for the last steps of QTL map-based cloning. Thanks to the different releases of the B73 genome sequences, we targeted gene-rich low copy regions using specific Long-Range PCR amplifications, on a QTL for which the confidence interval was narrowed down to 500kbp by classical QTL map-based cloning. This QTL contains 10 gene-rich regions accounting for 150Kbp on the B73 genome. Eighty one Long-Range amplicons, from 2 to 6 Kbp, have been amplified on 12 genotypes from diverse genetic origins. Depending on the genotypes, from 30 to 93% of the B73 designed amplicons have been successfully amplified allowing deep sequencing by 454 FLX Titanium followed by sequence assembly/mapping on B73 and SNP discovery. High throughput genotyping of these SNPs on a 347 lines maize diversity panel is in progress. Concurrently, a targeted-genotyping has been done, through Sanger amplicon re-sequencing, on the same 347 panel, on candidate genes chosen for their interesting annotations among the 10 gene-rich regions of our QTL. The preliminary association mapping results have highlighted interesting candidate genes that could be used in Marker Assisted Selection for maize yield improvement. This research was supported by Genoplante through the French National Research Agency (ANR).

Funding acknowledgement: Genoplante French National Research Agency

P233

Research Strategies Towards Improvement of Nitrogen Use Efficiency in Maize

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Nitrogen (N) is one of the most important nutrients influencing plant development and yield. Especially in maize high amounts of fertilizer are used as no negative impacts of high N exist towards quality or resistance. On the other hand economical and ecological reasons limit the input of N-fertilizers. The ability of a maize genotype to produce superior grain yields under low soil N conditions is defined as its N-use efficiency (NUE). The aim of the project is to identify and characterize important quantitative trait loci (QTL) and candidate genes for NUE in maize and to apply the results in practical breeding.

A QTL-analysis with doubled haploid (DH) lines derived from two maize genotypes contrasting in their NUE resulted in identification of two QTL on the chromosomes 8 and 10 specific for grain yield under low N input. Near isogenic lines were developed for these two QTL-regions, validated for the presence of the QTL, and used for further analysis. Focus on this work are (a) the development of molecular markers for the finemapping of the QTL-regions and linking them to the physical maize map. (b) Large scale transcriptome expression studies to identify putative candidate genes for the trait. (c) As grain yield is an expensive and time-consuming trait a hydroponic system was established to screen for root characteristics related to NUE in an early developmental stage.

Funding acknowledgement: KWS Saat AG

P234

Resolving the genetic architecture of resistance to *Aspergillus flavus* in maize

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Aspergillus flavus causes Aspergillus ear rot of maize and contaminates developing and mature kernels with aflatoxins. These potent toxins cause liver cancer, nutritional interference and immunosupression when consumed by humans and animals. One method of control is to exploit natural variation for resistance to aflatoxin accumulation in maize. Unfortunately, low heritability values (h^2) for resistance to aflatoxin accumulation make the use of host resistance a difficult task. Further dissection of the trait and its genetic components is necessary. In order to identify regions of the genome associated with resistance to both aflatoxin accumulation and fungal growth, we are studying the B73 x CML322 population of recombinant inbred lines by field and in vitro inoculation assays. In addition, to reduce the levels of noise produced by the environmental factors, a panel of selected near-isogenic lines from the resistant inbreds Tx303, CML52 and CML322 are also being evaluated. Results from two years of field experiments and three years of in vitro assays on these materials support the presence of resistance QTL on bins 4.08, 6.07 and 7.02. In addition, preliminary QTL meta-analysis combining results from 10 studies with different parents indicates that, despite the variability of the trait, a few QTL, such as those located in chromosomes 4.08, 5.03 and 7.01, are consistently identified.

P235

Selection of SSR molecular markers to search for QTLs associated with agronomic traits of grain and forrage maize

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The Agricultural Research Center of Mabegondo (CIAM) is located in the northwest of Spain. This center have been working on maize genetic breeding for the past 30 years using traditional selection methods in order to obtain lines and hybrids adapted to this area. These methods use a source of phenotypic expression and variability like selection criterion. The identification of molecular markers associated with loci that encode both qualitative and quantitative characteristics (QTLs) give us the possibility of marker-assisted selection (MAS) which combine phenotypic and genotypic variability as a source of information on the variability. The genetic variation obtained with molecular markers associated with trait of interest is used as a selection criterion by MAS.

Among the molecular markers used for this selection we have selected the SSR because of its ideal characteristics for this purpose such as high degree of polymorphism, codominant, with Mendelian inheritance, selectively neutral, frequent in the genome, high reproducibility and not influenced for the environment. In CIAM we have an initial collection of 240 SSR markers for maize, they had been chosen from the databases (http://www.maizegdb.org) and are distributed as evenly as possible along the 10 chromosomes. These markers are used for search and obtain QTLs associated with traits of interest for forage and grain maize. An initial project has tested the polymorphism of these markers in 140 lines of CIAM maize germplasm bank. Nowadays we have used polymorphic markers in a two interesting lines cross-F2 for agronomic traits in order to obtain the necessary QTLs for MAS.

Funding acknowledgement: National Institute of Research and Agrarian Technology and Food (INIA)

P236

Stability assessment of hybrids in Multi-Environment trials without replicates.

(submitted by Fabiano Pita <fpita@dow.com>)
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Corn breeding programs optimize resource allocation by maximizing the number of entry x location combinations. This allows breeders to evaluate more entries in more locations with the same amount of resources. In contrast, it creates great challenges regarding the evaluation of genotype by environment interactions (GE). The aim of this work was to identify a statistical methodology that would allow stability assessment of entries in an experiment using field trial data in which entries are not replicated in each location. Mixed model solutions were used to calculate the IQR% (inter-quartile range percent) statistics together with the average rank of entries over locations. Indexes combining these two measurements were also investigated. The results show that IQR% and average rank seem to provide a good indication of both stable and high yielding genotypes for a given set of locations.

P237

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P238

The Assessment of Gametophyte Factors in North Carolina Inbred Lines

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With the increasing demand for organic food products there is also a requirement for the implementation of crossing barriers in field crops, to prevent contamination of organic food material with the genetic material of transgenic cultivars. In maize one of the ways that crossing barriers can be utilized is with the increased use of Gametophyte factors (GA). Most GA alleles are found in tropical lines as they were unintentionally used to maintain desired maize color, or texture. Tests have been conduced for North Carolina State inbred lines with tropical backgrounds, some of which have had GA factors maintained through the inbreeding process. These lines carry different alleles that can be broadly categorized into Ga1-S, Ga1-M and ga1, though there are varying alleles and levels of dominance. The Ga1-M allele is a promiscuous GA factor, making crossing inhibition difficult. One of the continuing issues is a conversion of the Ga1-S alleles into Ga1-M or ga1 alleles when GA factors are in heterozygotes. The conversion rate of homozygotes is very low or non-existent making the process in heterozygotes hard to explain. Work also has been done to define tropical maize populations which may contain these factors; it has been shown that they exist at moderate frequency in Mexican hybrids. More work is needed to determine which of these allelic combinations are most stringent in hybrid crosses, so that these North Carolina lines can be developed into hybrids for organic field corn production.

P239

Transforming corn from a commodity crop to a higher-energy, multipurpose biofuel crop.

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The two predominant U.S. alternative transportation fuels in use today are ethanol, which is distilled from corn *(Zea mays)* and biodiesel, which is produced from soybeans *(Glycine max)*. We propose that a higher net energy balance can be obtained by the production of both ethanol and biodiesel from a single corn crop.

The Korean High Oil (KHO) is a previously uncharacterized corn germplasm, which was determined to have oil content of at least 20% compared to 3.5% for normal corn. The high oil is accounted for by a 2-fold increase of oil in the embryo and a 3-fold increase in embryo size . The doubling of oil in the embryo and a higher oleic acid level appears to be due to a unique allele of the *DGAT* (Diacylglycerol acyltransferase) gene, which produces a protein with an extra phenylalanine at position F469 (Zheng et al., 2008). A major QTL *(oil6)* that explains 26.62% of oil variation was mapped to chromosome 6 near *DGAT* and a major QTL *(sta9)*, which explains 25.22% of starch variation was mapped to chromosome 9 near *Waxy1*. An Affymetrix microarray was used to compare gene expression levels between KHO and A619. We found over 1,000 genes with at least 2- fold change between the two parental genotypes and ~500 genes with at least 5- fold change.

Two biorefining models were evaluated in their ability to separate the germ from the endosperm and extract oil from the germ. A wet milling degermination process was found to be the most favorable method to maximize recoverable oil from KHO. An economic workbook model was developed showing the high value of the KHO germplasm. This model allows the comparison of economic gains of various technologies to separate oil from starch and produce multiple products from locally adapted commercial varieties containing the KHO germplasm.

Funding acknowledgement: Discovery grant from the University of Minnesota Institute on the Environment (IonE), Minnesota Corn Growers Association

P240

Tropical Rust Molecular Breeding in Corn

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Tropical rust is a fungal disease caused by the pathogen *Physopella zeae* previously classified as *Angiopsora zeae*. The disease is characterized by the formation of small round yellow pustules on the upper surface of the corn leaf. There are two other rust diseases that occur in corn, Common rust caused by *Puccinia sorghi* and Southern rust caused by *Puccinia polysora*. Tropical rust is the most recent and troublesome of the rusts since it is greatly adaptable to new environments; it can spread rapidly and is highly aggressive killing the plant in a short time. The most effective and most preferred method of control for tropical rust is the planting of resistant hybrids. Markers that are genetically-linked to resistance to the tropical rust loci, provide an effective method for selecting varieties with increased resistance to tropical rust in breeding programs. PHS6Y is an inbred developed using a pedigree selection scheme at Itumbiara Research Center. PHS6Y was elected as the donor parent for the initial backcrossing program into four inbred lines.

We have developed molecular markers associated with the resistance to Tropical Rust trait. Any BACs that assembles to the contiguous DNA on the corresponding physical region can house marker loci associated with the tropical rust resistance trait. Resistant and susceptible lines were genotyped by Sanger re-sequencing of genomic targets. The targets were PCR-amplified utilizing available public genome sequence as reference for primer design. The public sequence corresponds to the inbred line B73 and was obtained by a BAC minimum tiling path strategy.

P241

Unraveling a flowering time QTL on chromosome 8

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A region on chromosome 8 has been described as one of the major QTL involved in flowering time in maize. Studies using the Nested Association Mapping population (NAM) suggest the presence of allelic series for flowering time at this locus, validating the previously described *Vgt1* allele (Salvi et al., 2007) present in northern germplasm for the early effects and identifying SNPs at *ZmRap2.7* associated with late flowering. With this work we are trying to validate the hypothesis of the existence of different functional alleles controlling distinct effects at the QTL. New genotypic data is available on the 282 lines of the association panel, including NAM population parents, and it allows a deeper study of the genetic linkage and the haplotypes in the region. NAM-Genome Wide Association Study (GWAS) for flowering time found SNPs associated with both positive and negative effects in the region. Fine mapping is in progress using different sets of near-isogenic lines (NILs) and heterozygote inbred families (HIF), representing both early and late backgrounds. Analysis of new data suggests the presence of multiple genes and alleles involved in flowering time in the region and reveals linked sites more than 300 Kb away from the initially identified SNPs. Further investigation is needed to confirm the number of genes and the richness of the allelic series in this complex region.

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P242

Whole Genome Resequencing for crop improvement

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Obtaining the genome sequence of a wide range of individuals of a species will generate vast amounts of informative datasets and enable the rapid discovery of much greater genome-wide sequence variation than has been identified previously. Tremendous advantage will be in the identification of trait genes that are associated with phenotypic characters. It can be achieved through looking for associations between phenotypes of interest and the DNA sequence variants in an individual's genome. With the decreasing cost of sequencing, the genetic maps of many species are getting increasingly dense and an individual's genotype can be determined at the positions of hundreds of thousands of single nucleotide polymorphisms, a great improvement for plant breeding and selection. Also, a wealth of knowledge will be gained from comparative genomic analyses within and across species, as how plants grow, function and survive different ecological conditions and various environmental stresses.

Whole genome resequencing approach has been successfully used in sweet sorghum, rice and soybean studies. Genes related to growth, architecture, maturity, productivity or resistance have been identified and can be further applied in breeding programs. Similar study has also been carried out in maize for whole genome resequencing of several maize inbred lines. A large number of SNPs and InDels were identified. Hundreds of genes that are present in one haplotype but absent in another were detected. More than 100 large chromosomal intervals with low-sequence-diversity represent putative selective sweeps which may be related with domestication. Limited amounts of intra-chromosomal recombination during pedigree breeding were identified. Whole genome resequencing will have far reaching implications for improving breeding strategies and plant varieties to meet the world's growing demand on plant production.

P243

Accessing the transcriptional activity of *Helitron*-captured genes of maize

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The *Helitrons* of maize are known for their propensity to capture and mobilize gene fragments into different regions of the genome. Sometimes, the captured gene fragments are transcribed, intertwining coding regions of different genes. This may give birth to new genes with novel domains. The promoters driving the transcription of the captured genes, in some cases, are located in the flanking sequence, the extent of this process and its impact on the host genome remains undetermined. In this report, we wrote a script using python programming language, which utilizes conserved termini of known maize *Helitrons* to discover putative, high quality *Helitrons* in the maize genome. Those exhibiting +/- polymorphism in different inbred lines and that harbor gene fragments and display strong EST evidence of transcription were further selected for manual annotation and validation by RT-PCR analysis. Our data provides evidence in support of *Helitrons*, which in some cases may propel evolution of novel genes and identify potential *Helitrons* in which the promoter driving the transcription of the captured genes is located inside the element. We also identify a *Helitron* that may have captured a full-length, active gene. Resulting data provide evidence in support of promoters located within *Helitrons*, which drive expression of chimeric genes.

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P244

Estimates of conversion tract lengths in dimorphic heterozygotes

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Maize intragenic recombinants (IGRs) carrying a parental or noncrossover (NCO) arrangement of flanking markers generally arise from conversion events. The stretch of DNA that is transferred during a gene conversion event, called the conversion tract, can vary in yeast from a few hundred bases to more than 12 kb. Maize IGRs are generally recovered from polymorphic heterozygotes between alleles differing at many heterologies other than the two between which recombination is being measured. Because NCO IGRs from such heterozygotes are rare in maize, only a few conversion tracts have been measured to date and their minimum length has ranged from 0.5 to 3 kb (1). Surprisingly, NCO convertants occur frequently among *Bz* IGRs from dimorphic heterozygotes between alleles that differ only at the the two heterologies between which recombination is being measured (2). Estimating conversion tract lengths in dimorphic heterozygotes, a basically homozygous configuration, is a problem because markers cannot be introduced by transplacement. We have developed a method to measure the minimum-maximum lengths of conversion tracts that utilizes unselected *Ac* excision footprints at various distances from *bz*. So far, we have been able to establish that only 2 out of 44 conversion tracts extend proximally to a marker located 3.3 kb away in the next upstream gene and none extend distally to a marker located 6.8 kb away in the next downstream gene. Not surprisingly, conversion tracts never cross a 100-kb proximal retrotransposon cluster into the next gene island. Only 25% of tracts involving a site 0.5 kb downstream are larger than 0.5 kb and 75% are smaller than 1.2 kb, a value at the low end of the 1.0- to 2.0-kb yeast average (3). Our data suggest that conversion tracts in dimorphic heterozygotes may be considerably shorter than those reported previously in polymorphic heterozygotes.

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P245

Analysis of *Ac/Ds* transposons in the maize inbred lines B73 and W22

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Although *Ac/Ds* transposons are known to transpose all over the genome, they have an inclination to jump into or near genes. Thus, *Ac/Ds* elements are an invaluable resource in maize gene knockout studies. To characterize the background of *Ds*-like elements in an inbred background lacking *Ac*, we have analyzed the spectrum of *Ds*-like elements in the recently sequenced B73 genome. We restricted our analysis to elements possessing 11-bp terminal inverted repeats and flanked by perfect 8-bp target site duplications. Among these, there is one inactive 4.5-kb internally deleted *Ac*-like element. All other elements are much shorter: there are 221 type 1 *Ds1* elements and 200 type 2 *Ac* deletion derivatives. *Ds*-like elements occur in all ten chromosomes, in numbers proportional to chromosomal length. Among the type 2 *Ds* elements, 23 are related to the 1.3-kb *Ds2*, 25 have homology to the internal transposase of *Ac*, 30 are composed of only truncated subterminal regions of *Ac*, and the rest share similarity with only the 3' end of *Ac*. Twenty-four type 1 and seven type 2 *Ds* elements appear to have inserted into genes. The size of the type 2 elements ranges from 600 bp to 20 kb. *Helitrons*, LTR retrotransposons, and full length cDNAs can be found embedded in the larger elements. We are also using next-generation sequencing technology to characterize the spectrum of *Ds*-like elements in W22. A detailed comparison between the SOLiD-generated W22 data and the B73 genome sequence data will be presented.

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P246

Cytoplasm-Induced Paramutations In Maize

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Maize lines with one and the same nuclear genomes in the *N*-, *T*- and *S*-cytoplasms were obtained by androgenesis *in vivo* (male partenogenesis). According to this technique, in the androgenesis-inducing line, the nucleus of the egg cell sometimes does not take part in fertilization and is replaced by the sperm nucleus; as a result the haploid and diploid plants occur, which contain nuclear genome of paternal line in the cytoplasm of maternal parent. Such androgenic plants were pollinated by the line-donor of the nuclear genome, which had nuclear genes determining brown plant color (*aBPlR*). In the new nuclear-cytoplasmic combinations heritable changes of plant color (from brown to green), plant height and ear length occurred. In some nuclear-cytoplasmic combinations the changes of all these traits were found, while in the others only plant height changes were observed. Sometimes, these changes were observed already in the original haploid plant while in the others they were found in subsequent generations, which were obtained by pollination of original haploid with the pollen of the line-donor of the nuclear genome. The proportion of plants with changed traits varied from single plant up to the 100%. Color changes may occur sharply or gradually during several generations and were stable for more than 10 generations. In test-crosses of plants with changed color (green) to the line-donor of the nuclear genome (brown), i.e. after transfer of changed genome to original cytoplasm, all the progeny maintained green plant color. In the self-pollinated progeny of such crosses segregation of brown plants was not observed. Consequently, phenotypic changes found in our androgenic lines are quite similar to paramutations of *B* and/or *Pl* genes. These data suggest significant role of cytoplasm in initiation of paramutations and in occurring of heritable changes in numerous individuals in plant populations.

P247

Examining Histone Modifications in the Pericentromere of Maize

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Pericentromeres are functionally defined as the heterochromatic regions flanking centromeres and the primary site of cohesion formation important for the binding of sister chromatids. The deposition of cohesin appears to be epigenetically regulated, and in plants is poorly defined since heterochromatin tends to be distributed throughout the genome. We hope to identify modifications of the N-terminal tail of Histone 3 that can be used to differentiate and define the functional pericentromeric regions. We are also interested in how pericentromeric marks, such as histone methylation, are maintained over evolutionary time. As a first step we have focused on the maize retroelements Cinful and Zeon, which are abundant and distributed throughout the maize genome (including centromeres, pericentromeres, and euchromatin). Transposon display was used to genetically map over 100 Cinful and Zeon elements throughout the genome, emphasizing the pericentromeric region of chromosome 2. A chromatin immunoprecipitation transposon display (ChIP-TD) method is being used to map the distribution of histone methylation to these Cinful and Zeon elements. To assess the stability of histone modifications marks over time, we will score the same Cinful and Zeon markers by ChIP-TD in 53 diverse inbred lines. Our hypothesis is that histone marks diverge more rapidly than DNA sequence, and that the stability of the histone marks will vary with respect to where Cinful and Zeon elements are relative to centromeres. Our study will allow us to identify epigenetic marks that are prevalent in pericentromeric regions, and to begin an investigation of how pericentromeric regions evolve.

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P248

Functional characterization of the *nfc102* maize gene

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The FP7-EU-funded project named "AENEAS" (Acquired Environmental Epigenetics Advances: from Arabidopsis to maize) aims to unravel the mechanisms of epialleles formation in response to environmental stresses in Arabidopsis and maize. In the frame of this project, we are investigating the targets and the epigenomes of specific epi-regulators known for interacting with environmental cues, including the maize *nfc102* gene.

The *nfc102* gene encodes for a WD-repeat protein belonging to the Multicopy suppressor of IRA (MSI) family. In both Arabidopsis and maize, the MSI family comprises five members, which on the basis of sequence homology can be grouped in two separate classes. The maize *nfc103*, *nfc104* and *nfc108* genes are members of the first group and encode for putative orthologs of the Arabidopsis *MSI1*-like gene, which is involved in the epigenetic control of reproductive development. The maize *nfc101* and *nfc102* genes are in the second group and are related to the Arabidopsis *FVE* gene, which plays important roles in the regulation of flowering time and in the control of epigenome stability.

In order to highlight the functional role of the *nfc102* we have obtained maize antisense and RNAi transgenic mutants with down-regulation of *nfc102* expression. Quantitative RT-PCR experiments showed that transcript levels of both *nfc102* and *nfc101* were reduced in V2/V3 leaves and in tissue enriched in the shoot apical meristems of antisense and RNAi mutants compared to wild-type. Preliminary phenotypic analysis, together with detailed expression profile studies, will be presented as a first step towards the functional characterization of the maize *nfc102* gene. The strategy for genome-wide analysis of transcriptome and epigenome changes in *nfc102* mutant will be also presented.

P249

Poster removed

P250

Identification of natural variation in DNA methylation in maize inbred lines using a genome methylation analysis

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Previously, genetic variation among individuals was considered only in terms of alterations in the primary nucleotide sequence due to mutations or deletions by aberrant gene recombination. However, it is now known that epigenetic factors such as DNA methylation and histone modifications can play a fundamental role in generating alternative states (epialleles) of gene expression among biological strains or individuals in a population. In maize plants, spontaneous epialleles have already been identified at some loci, such as those which encode transcription factors that activate the biosynthesis of flavonoid pigments. In order to gain further insight into rules governing both the establishment and propagation of epialleles in maize we searched for target regions showing methylation differences among individuals of the Mo17 inbred line. Using a methylation sensitive amplified polymorphism (MSAP) approach the methylation state of approximately 1000 DNA sequences was investigated in genetically identical individuals at different developmental stages. Here we show that 1% of the inter-variability of DNA methylation identified in our study is meiotically inherited over at least five generations. Furthermore, we observed that novel methylation differences arise in each generation largely in a parent–of-origin-dependent manner. Evidence providing a possible explanation for alternative phenotypes observed in the progeny of long-term inbred lines, through the production at each plant life generation of novel epigenetic states, are supported by our study.

P251

Identification and prediction of miRNAs from maize seedling roots responsed to lead (Pb) stress using deep sequencing

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Recently, microRNAs (miRNAs) as one class of noncoding, short, regulatory RNAs, were reported to play big roles in plant development and stress response. In order to indentify the miRNAs related to heavy metal stress, deep sequencing approach was used for genome-wide prediction of known and novel miRNAs in maize seedling roots responsed to Pb stress, which led to the discoveries of 92 known miRNAs and 378 novle miRNAs. 92 known miRNAs, belonging to 18 miRNA families are conserved among plant species. 378 novle miRNAs, belonging to 185 miRNA families and thereinto, 20 miRNA families are conserved at least in two gramineous species. Target prediction revealed 54 and 489 potential proteins for known and novle miRNAs. For known miRNAs, most of these targets are transcription factors and some of which have been validated in regulation of plant development and stress response in Arabidopsis and rice. Among the 489 targets of novel miRNA, 453 targets are functionally annotated and the other 36 records are unknown proteins. Functional prediction indicate most of target genes are involved in diverse biological processes such as metabolism, transcription regulation, ion transport, stress respones, signaling pathway and so on. Many targets, including ABC transporter, P-type ATPase, bZIP and metal transcription factors are predicted to be directly involved in plant tolerance to Pb stress. These results can be guide for further understanding the tolerant mechanism mediated by miRNAs in maize seedling roots responded to Pb stress.

P252

Mapping and phylogenetic analysis of Mu transposon sequences in maize and teosinte inbreds

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Our bioinformatic analysis of the B73 maize genome has identified over 150 Mu transposons. Because B73 and other maize inbreds are thought to lack active transposases (MuDR), their complement of Mu elements is expected to be relatively stable. Phylogenetic analysis indicates that the majority of the Mu transposons in the B73 genome belong to a diverse clade of Mu12-related elements. Mu12, initially described by Dietrich et al. (2002), is distinct from the 'canonical' Mu's more commonly employed in genetic studies. Here we have found that in addition to the predominance of Mu12-like's among the Mu's of maize and teosinte, there is a smaller Mu10-like group, which is closely related to the canonical Mu9 (MuDR) transposases. We have utilized PCR and 454-based Mu-flank sequencing to characterize the Mu transposon insertions in Mo17 and W22 maize inbreds, as well as five inbred teosinte lines (courtesy of J. Doebley). We have found that most of the Mu transposons in a given inbred are unique to that genome. Analysis of these Mu transposon profiles and the conserved regions in Mu sequences provides new insights into the evolutionary history of Mu elements in teosinte and maize. The presence of many Mu insertions in genic DNA suggests that they could potentially influence gene expression and consequently the phenotypes of maize inbred lines.

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P253

Mechanism and Genetic Impacts of Transposon Ac/Ds-Induced Rearrangements in Maize

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We have shown that alternative transposition involving a pair of directly oriented Ac/Ds transposon termini can induce deletions or inverted duplications; and that transpositions involving a pair of reverse-oriented Ac/Ds transposon termini can induce deletions, inversions, translocations and duplications. Both configurations can induce chromosome breakage, and the frequency of breakage shows an interesting relationship to inter-element distance. We are now focused on a series of partial chromosome duplications (segmental duplications) generated by Ac/Ds alternative transposition.

Segmental duplications are important contributors to the diversity of natural genomes. However, very little is known concerning how duplications are generated, and their immediate effects on gene expression and recombination. This project examines the potential role of transposable elements in generating segmental duplications. Our aim is to isolate and characterize a series of partial chromosome duplications in corn generated by Ac/Ds transposable elements. Duplication structures will be confirmed by cytogenetic methods, and the endpoints will be sequenced to determine how DNA replication and genetic recombination are involved in forming duplications. Finally, the duplications will be studied to determine their impacts on recombination, gene expression, and plant phenotype.

This research was supported by NSF award 0450243 (Collaborative Research: Comprehensive Molecular, Genetic and Cytogenetic Analysis of Transposon-Induced Chromosomal Rearrangements in Maize) to T. Peterson, J. Zhang, and D. Weber; and by NSF award 0923826 (Mechanism and Genetic Impacts of Transposon-Induced Duplications in Maize) to T. Peterson, J. Zhang, D. Weber, and D. Nettleton.

Funding acknowledgement: National Science Foundation (NSF)

P254

On the Role of RNA in Centromere Chromatin

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While many of the protein components of centromeres have been identified, and centromeres have been mapped to specific genomic loci, fundamental questions about centromere identity and function remain unanswered. The central goal of our research is to understand the contribution of chromatin-associated RNA to centromere regulation and function. As an initial step, we are surveying the repertoire of RNA associated with normal centromeres by sequencing from several maize tissues. We are preparing to use the knowledge gained from normal centromeres to investigate special centromere cases where the influence of DNA, protein, and RNA factors can be teased apart, in the hopes of understanding the interdependence between these factors and the specific function of RNA in centromere chromatin.

Funding acknowledgement: National Science Foundation (NSF)

P255

Paramutation is regulated differently at distinct loci

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Our long term goal is to understand the molecular mechanisms mediating paramutation, which occurs when specific homologous sequences communicate in trans to establish a meiotically heritable change in gene expression. Our current data indicate that paramutation involves an RNAi transcriptional silencing pathway that establishes heritable changes in chromatin structure. Paramutation at the *b1* (*booster1*) locus requires MOP1 (RDR), MOP2/RMR7 (NRPD2/E2), and RMR6 (NRPD1). Maintenance of silencing associated with *b1* paramutation requires the above proteins and RMR1(SWI/SNF2-like factor). Our genetic experiments demonstrate that similar to *b1*, paramutation at *p1* (*pericarp color1*) requires MOP1 and MOP2 (Sidorenko and Chandler 2008, Sidorenko et al., 2009). In contrast to *b1* paramutation, maintenance of silencing associated with *p1* paramutation is not immediately disrupted by deficiencies in MOP1, MOP2, and RMR1. No detectable increases in pigmentation are observed after multiple consecutive generations of exposure to the *Mop2-1* and *rmr1-1* mutations and only partial up regulation is observed after two or more generations of exposure to the *mop1-1* mutation (Sidorenko and Chandler 2008, and unpublished data). These results clearly demonstrate that while *p1* paramutation depends on RNA-mediated silencing mechanisms, maintenance of silencing is likely to involve other mechanisms that are only partially dependent on RNA signals, and therefore likely to be regulated by a distinct set of factors. To uncover factors required for *p1* silencing a new EMS mutagenesis screen is in progress.

Funding acknowledgement: United States Department of Agriculture (USDA)

P256

Parental line dependent expression of specific alpha-zein isoforms in maize endosperm depends upon gene methylation state and genotype-specific maternal factors

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Maize genotypes accumulate both different types and variable amounts of alpha-zein polypeptides in the endosperm. Two-dimensional fractionation, by size and charge (2D), resolves the heavy-zein (zH) and light-zein (zL) subclasses into several isoforms, with profiles specific for each maize line. Seven *opaque2* (*o2*) genotypes with different 2D zein patterns were considered in this study. We investigated the expression of the zH subset in the F1 of both inbreds and their reciprocal crosses of four *o2Italian* genotypes characterized by the presence of specific H isoforms (Hp lines), and three *o2R* genotypes, which are nearly null for their presence (Hn lines). Among the 24 reciprocal crosses analyzed, two Hn lines as maternal parents caused the silencing of specific zH polypeptides from only two of the Hp lines. This unidirectional silencing was thus cross-line specific, and identified these maize lines as paternal–parent silenceable and as maternal-parent repressor for the zH trait. Clones of the zH paternally-silenceable zein genes (*Pszg*) were obtained. *In vivo* and *in vitro* analyses of total RNAs from endosperms of the parental lines and their reciprocals indicated the absence of the zH transcripts in one of the two reciprocals (repressed cross) versus their presence in the other. PCR analyses with *Pszg*-specific primers revealed the presence of the *Pszg* sequences in the genome of the Hp line but their absence in the Hn repressor line. The *Pszg* were found to be hypermethylated in F1 kernels obtained from crosses involving either maternal repressor or maternal non-repressor lines, indicating that hypermethylation *per se* is not sufficient to determine *Pszg* transcriptional silencing. Genetic analyses of F2 progenies and back-crosses indicated that *Pszg* sequences maintain their repressible state (hypermethylation) while the suppression activity of the Hn-line is lost when HnHp hybrid is used either as maternal parent or in self pollination. The overall results indicate that the unidirectional silencing depends upon the combination of both genotype-specific maternal factors and the methylation state of the *Pszg*.

Funding acknowledgement: Consiglio Nazionale delle Ricerche (CNR)

P257

Sequence acquisition by *Mutator* elements in maize

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Mutator (Mu) elements belong to DNA transposons and are the most active and mutagenic family of transposons in plants. Non-autonomous *Mu* and *Mu*-like elements (MULEs) have been shown to capture gene fragments, which may result in the creation of novel genes. *Mutator* and MULEs that acquire gene fragments are referred to as Pack-MULEs. However, little is known about the mechanism of sequence acquisition by these elements and whether this is a currently on-going process in the maize genome. To address this, a genome-wide, nested PCR-based screen was developed to detect sequence variation, which may reflect novel acquisition events, among individual elements of the *Mu1* subfamily. With this approach, different types of *Mu1* variants involving rearrangements and deletions were detected. In addition, three variants harboring gene fragments not previously associated with *Mu1* sequence were found. The gene fragments are highly similar to existing genes in the maize genome indicating that acquisition of new sequences by *Mu1* elements is a relatively recent process in maize. The novel acquired regions in the variants include both intron and exon sequences, with none of them derived from intergenic regions. A comparison between the variants and the putative parental genes indicates no deletions and rearrangements in the acquired regions. Given that genes only represent a small fraction of the maize genome, these results suggest that the sequence acquisition by Pack-MULEs is biased toward genic regions. Moreover, the initial sequence acquisition of Pack-MULEs is likely a process with high fidelity and rearrangements of captured fragments may occur after acquisition.

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P258

The Maize Gene Machine in Biogemma : an overview

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For over ten years now Biogemma has been using the so called « Maize Gene Machine », a mutagenesis population made up with 43,544 plants in which the Mutator transposons inserted randomly. The development of this functional genomics tool has been partly funded by the French initiative Genoplante. New insertions of interest for Biogemma's programmes are regularly identified by reverse genetics; the selected plants are backcrossed in an elite genetic background, then selfings allow phenotypic scorings in either hybrid or per se value. Since 2000, 372 genes have been tagged by Mu over the 563 studied, which means a success rate of 66%. Until recently the Maize Gene Machine has been mainly used for gene functional validation (screen for mutants in regulation pathways: ABA for resistance to drought, lignin synthesis for digestibility, etc). Now the search for mutants as "products" to be quickly usable in breeding is preferred, that's why an effort is made to select in first the genes for which a mutation may induce a favorable effect. To improve our efficiency, FSTs have been systematically sequenced to identify all the mutated genes in our collection. This is a pilot experiment that is expected to significantly speed up mutant discovery. Fundamental knowledge on our Mu population genetic material has also been increased. The Mu composition has been analyzed, and the influence of backcross and self design on the transposable activity has been tested. This epigenetic approach has also been studied in the field, by crossing some mutant lines with a Mu-killer line. Thanks to our experience on this resource, we have been able to settle optimized processes in both the field and the laboratory, which allows us to work and valorize them more efficiently (discarding of MuDR insertions, length of the introgressed genomic region around the mutation, etc).

P259

The maize *Unstable factor for orange1* is required for maintenance of histone modification at *pericarp color1* gene

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The *Unstable factor for orange1 (Ufo1)* has been characterized as a dominant trans-acting modifier of a tissue-specific allele of *pericarp color1* gene (*p1*). The *p1* gene encodes a Myb transcription factor and regulates the accumulation of phlobaphenes (flavonoid pigment) in floral organs. *P1-wr* (white pericarp and red cob glume), allele of *p1* was shown to have a multicopy gene structure that is transcriptionally regulated by a mechanism correlated with DNA hypermethylation and results in tissue-specific pigmentation. *Ufo1-1* epigenetically regulates *P1-wr* gene expression, leading to enhanced accumulation of phlobaphenes in pericarp, cob glumes, husk, silks, leaf sheath, and tassel glumes. We have previously demonstrated that *p1-Ufo1-1* interaction leads to DNA hypomethylation of *P1-wr* allele. Using chromatin immunoprecipitation assays (ChIP), we further identified that the wild type *ufo1* is required for maintenance of the histone modification of *P1-wr* allele. Two repressive histone marks H3K9-me2 and H3K27-me3 are both decreased at the *P1-wr* in the presence of *Ufo1-1* mutation. In addition, these histone marks are reduced at *Copia* retrotransposon. Our results suggest that DNA methylation and histone modification are both associated with *P1-wr* tissue-specific expression and retrotransposon silencing. Moreover, *ufo1* is also involved in the maintenance of the silenced state of other single copy *p1* epialleles, *P1-rr'* and *P1-pr*^{TP} (Sekhon et al., unpublished). The *P1-rr'* allele participates in paramutation and this interaction involves DNA methylation dependent mechanism. However, *P1-pr*^{TP} shows a typical gene suppression phenomenon and its interaction with *Ufo1-1* does not affect DNA methylation at *p1* sequence. We will perform ChIP assays to identify histone modification difference that may operate during interaction of *Ufo1-1* with these single copy *p1* alleles. These results will provide a better understanding of the role of *ufo1* in tissue-specific gene expression and paramutation in maize.

1. Sekhon, R., Sidorenko, L., Chandler, V., and Chopra, S. A maize *unstable factor for orange1* mutant reactivates two differentially paramutagenic epialleles of *pericarp color1*.

Funding acknowledgement: National Science Foundation (NSF)

P260

The spectrum of mutations produced at a locus by the autonomous transposon *Ac*

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The autonomous transposon *Ac* is known to predominantly cause mutations at a locus by either frequent excisions that create footprints or rare internal deletions that produce new *Ds* elements. These two types of mutation usually share the same phenotype since excisions and internal deletions of *Ac* lead mostly to the formation of nonfunctional alleles. However, it has not been possible in the past to examine the entire spectrum of mutations that Ac can generate at a single locus because the common *Ac* excision footprints outnumber the other events by about 10:1. To eliminate these excision footprints from the collection of mutations to be analyzed, we chose the bronze mutable allele *bz-m39 (Ac)* that harbors an *Ac* element in the 5' UTR of the bz gene. Because the transposon footprints generated by *Ac39* excisions in the *bz* 5' UTR do not interfere with gene function, all simple excisions will produce purple (Bz') revertants, rather than stable *bz* derivatives. We are analyzing 73 stable bronze derivatives from *bz-m39 (Ac)* and, so far, have found eight different types of mutation, including: 50 new *Ds* elements, 1 immobilized *Ac*, 3 5'-adjacent deletions, 2 3'-adjacent deletions, 4 5'-fractured *Ac* , 2 fractured *Acs* (3' or 5') with an adjacent deletion, and 3 *Ac* excisions with an adjacent deletion. The most likely mechanism of origin of these different derivatives will be discussed.

Funding acknowledgement: Waksman Predoctoral Fellowship

P261

Uncovering the Methylation Dynamics at the Maize Flowering Time Locus *Vgt1*

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Flowering time is an important agronomic trait; so far, only a few loci involved in this phenomenon have been described at the molecular level in maize. The *Vegetative to generative transition 1 (Vgt1)* locus was positionally cloned on chrom. bin 8.05 after its localization as a major QTL (Salvi et al., 2007, PNAS, 104: 11376-11381). *Vgt1* corresponded to an upstream (70 kb) non-coding regulatory element of *ZmRap2.7*, an Ap2-class transcription factor which was shown to influence flowering time. A transposon (MITE) insertion was identified as a major allelic difference within *Vgt1*. One of the hypotheses is that *Vgt1* might function by modifying *ZmRap2.7* chromatin through an epigenetic mechanism. Therefore, we decided to investigate the methylation state at multiple regions of ca. 250 bp each, within *Vgt1* and the promoter of *ZmRap2.7*. Following digestion with McrBc, an endonuclease that acts upon methylated DNA, real time PCR analyses were performed on genomic DNA from near-isogenic maize lines carrying different combinations of late and early alleles at both loci. DNA was extracted from leaves sampled at 1-, 2- and 3-leaf stages of development. Preliminary results showed a trend of reduction of methylation from the first through the third leaf stage. However, no clear trend was identified when comparing the relative methylation level between the two alleles (N28 and C22-4/Gaspé) across the six target regions at *Vgt1*. The region closer to the MITE insertion showed a constant and very dense methylation level throughout leaf development and for both alleles. To go more into depth, additional genotypes and stages of development are actually being included into the analyses.

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