ABSTRACTS: PLENARY TALKS, REGULAR SESSIONS, WORKSHOPS, FOLLOWED BY POSTERS.

TALKS ARE ORGANIZED BY CATEGORY AND WITHIN CATEGORY, BY ORDER OF PRESENTATION.

Plenary Talk 1 7:30 PM THURSDAY MAR 15

Abnormal chromosome segregation: meiotic drive and neocentromeres
Dawe, R. Kelly (1); Hiatt, Evelyn N.(1); Yu, Hong-Guo(1); Mroczek, Rebecca J.(1); Lawrence, Carolyn J. (1); Muszynski, Michael G.(2) (1) University of Georgia, Athens, Georgia 30602, USA; (2) Pioneer Hi-Bred International Inc., Johnston, Iowa 50131, USA

Meiotic drive occurs when a chromosome or chromosomal segment is transmitted to progeny at frequencies higher than expected from Mendelian segregation. One example of meiotic drive is the preferential segregation phenomenon associated with Abnormal chromosome 10 (Ab10) in maize (Rhoades, 1942). Approximately 70-75% of the female gametes carry Ab10 after the chromosome undergoes meiosis with normal chromosome 10; a frequency that is significantly greater than the 50% expected under random segregation. This nonrandom segregation event is due in part to the activity of Œneocentromeres, which are knobs that have been induced to move poleward on the spindle in response to genetic information on Ab10. We have taken classical genetic approaches to analyze the functions required for meiotic drive and used high-resolution light microscopy to compare the molecular makeup and mechanisms of movement typical of normal centromeres and neocentromeres. There are several functions required for maximal meiotic drive, including what appear to be two independent neocentromeres systems with different affinities for microtubules. Neocentromeres do not interact with microtubules in the same manner as centromeres/kinetochores, and lack at least two key kinetochore proteins. Current studies are aimed at better understanding the mechanism of neocentromere motility and how it relates to the mechanism of normal chromosome movement.

Plenary Talk 2 8:15 PM THURSDAY MAR 15.

Evolution of breeding systems in crucifers: from outcrossing to autogamy in one step.

June Nasrallah, Cornell University, Ithaca, NY

Self-incompatibility (SI) is the major outcrossing mechanism in the family Brassicaceae. Molecular analysis of the Brassica S locus, which controls specificity in the SI response, has shown that this mendelian locus is a gene complex consisting of highly polymorphic stigma-expressed and anther-expressed genes that determine SI specificity in stigma and pollen, respectively. We recently initiated a phylogenetic study of self-incompatibility in other crucifers to gain insight into the origins of S-locus polymorphisms and to elucidate the genetic basis of transitions between outcrossing and self-fertilizing mating systems in this family. As a starting point for this study, we investigated the SI system of Arabidopsis lyrata, an outcrossing close relative of the self-fertile A. thaliana. The results of a comparative mapping study of the Brassica, A. lyrata, and A. thaliana S loci will be discussed in the context of the evolution of the Slocus and the function of SI specificity genes.

Plenary Talk 3. 7:30 PM SATURDAY MAR 17

Cell Biology of Maize Leaf Development.
Laurie Smith, U.C. San Diego La Jolla, CA USA

The cellular architecture of plant tissues reflects the patterns in which cells divide and expand during organ development. Taking advantage of the extremely regular cellular organization of the maize leaf epidermis, we have isolated a variety of mutants defining genes required for the spatial control of cytokinesis and cell morphogenesis during leaf development. The tangled gene is required for proper orientation of cytoskeletal arrays associated with cell division during the proliferative divisions that give rise to most leaf cells. This gene encodes a highly basic protein that can bind directly to microtubules and belongs to a family of proteins that are associated with the cytoskeletal arrays in dividing cells that are misoriented in tangled mutants. Three classes of mutants have also been identified that are specifically required for the proper orientation of asymmetric divisions in the maize leaf epidermis. Among these, the Discordia genes are required for guidance of phragmoplasts to appropriate cortical attachment sites, while Brick and Pangloss genes are required earlier for polarization of cells prior to mitosis. Brick genes are also required for normal epidermal cell morphogenesis - in brick mutants, the lobes on the margins of epidermal cells fail to form. Recent work suggests that formation of these epidermal lobes also depends on the establishment of cell polarity.

Plenary Talk 4 8:15 PM SATURDAY MAR 17

Genetic dissection of chloroplast gene expression: a tale of two gene families.
Barkan, Alice(1); Belcher, Susan(1); Carrier, Rosalind(1); Fisk, Dianna(1); Jenkins, Bethany(1); Ostheimer, Gerry(1); Till, Brad(1) (1) University of Oregon, Eugene, OR 97403

A central component of chloroplast differentiation and environmental adaptation is the regulated expression of the chloroplast genome. We have used genetic screens to identify nucleus-encoded factors that are required for the expression of subsets of chloroplast genes. The molecular cloning of several of these genes has provided a glimpse into the functions of two gene families that appear to be specific to plants. One family came to light in studies of nuclear genes involved in group II intron splicing in chloroplasts. Although group II introns are often referred to as "self-splicing", accessory factors are required for their efficient splicing in vivo. The maize genes crs1 and crs2 encode two such factors: CRS1 is required for the splicing of a single group II intron, whereas CRS2 is required for the splicing of numerous group II introns in the chloroplast. We have cloned crs1 and crs2 and initiated biochemical studies of their gene products. CRS1 and CRS2 are components of distinct ribonucleoprotein particles in the chloroplast. Although CRS1 and CRS2 are unrelated to one another, biochemical and 2-hybrid data indicate that CRS2 is found in a complex with proteins that are related to CRS1. The CRS1 family is represented by 16 predicted proteins in Arabidopsis, all of which include a 10 kDa repeated domain that is of ancient origin but unknown function. Other findings lead us to speculate that the ancestral 10 kDa domain functions in translation, and that it was recruited to facilitate the splicing of group II introns in chloroplasts, and possibly, mitochondria. We are now testing these ideas using biochemical, structural, and genetic approaches. The maize gene crp1, required for the translation and processing of several chloroplast mRNAs, is the founding member of a particularly large family of predicted plant proteins, represented by hundreds of genes in Arabidopsis. CRP1 contains 14 tandem copies of a "PPR" motif (Small and Peeters,2000, TIBS 25,46), a motif that resembles the TPR motif, a widely distributed mediator of protein: protein interactions. Many members of the PPR family are predicted to be localized to mitochondria and chloroplasts. We are using biochemical and reverse genetic approaches to elucidate the functions of CRP1 and other PPR proteins, with the goal of understanding the functions of the PPR motif and why this protein family may have expanded specifically in plants.

SESSION 1 8:30-10:10 AM FRI

T1 Ligand-dependent expression of MS45 results in the conditional regulation of male fertility in maize.

Unger, Erica(1); Cigan, A. Mark(1); Xu, Rui-ji(1); Kendall, Tim(1); Albertsen, Marc(1); Roth, Brad(2) (1) Plant Reproductive Biology, Pioneer Hi-Bred, Johnston, IA 50131, USA; (2) TTL, Pioneer Hi-Bred, Johnston, IA 50131, USA

A mutation in the maize Ms45 gene results in a male-sterile phenotype due to the absence of pollen. Cytological examination of microspores in ms45 mutant plants reveals that the cell wall of the developing male gametophyte is poorly formed, while genetic analysis suggests MS45 functions sporophytically. The Ms45 gene was isolated and complementation analysis determined that a transformed copy of the gene was able to fully restore fertility to ms45/ms45 mutant maize. MS45 protein was localized to the tapetum and maximally expressed in anthers at the early vacuolate stage of microspore development. Based on the temporal and spatial expression requirements of MS45, synthetic chimeric transcriptional activators were designed and expressed with a minimal gal:MS45 gene to test the ability of a multigene system to restore fertility. A high frequency of phenotypic complementation was observed using this approach when the C1-GAL4 or VP16-GAL4 activators were transcribed by promoters that expressed at a stage of anther development that precedes MS45 transcription. To develop a system to conditionally regulate male-fertility in maize, these transcriptional activators were modified by the addition of the ligand-binding domain of the corn borer ecdysone receptor generating ligand-dependant transcriptional activation of MS45. These chimeric receptor proteins were introduced with the gal:MS45 gene into mutant ms45/ms45 maize. In the absence of ligand, these plants were male-sterile. In contrast, application of an ecdysone agonist resulted in the induction of MS45 protein expression in anthers and restored male fertility to ms45/ms45 mutant plants.

T2 The male-sterile mutants of maize

Bedinger, Patricia(1); Chaubal, Raj(1); Anderson, John R.(1); Erickson, Ami N.(1) (1) Colorado State University, Fort Collins, CO 80523-1878, USA

We are attempting to identify all of the sporophytic genes specifically required for the successful completion of the pollen developmental pathway, in collaboration with Dr. Marc Albertsen at Pioneer HiBred. In addition, our laboratory has undertaken the structural analysis of all of the available male sterile mutants of maize. We have performed extensive allelism studies with the available mutants, and can now confirm the presence of 36 different male sterility loci, with at least 6 more to be confirmed in summer of 2001. Our structural studies have revealed an array of mutant phenotypes at the organ, tissue, cellular and subcellular levels. I will describe the mutant phenotypes of the different male sterile mutants of maize, from those that cause an altered anther fate to those that cause an extended developmental "stall" very late in pollen development.

T3 Regulation of meristem function by thick tassel dwarf1 and interacting genes
Running, Mark P. (1); Vollbrecht, Erik(1); Hake, Sarah(1) (1) UC Berkeley Plant Gene Expression Center, 800 Buchanan St., Albany CA 94710, USA

Plant meristems are responsible for balancing cell proliferation with the allocation of cells for organ initiation. Recessive mutations in thick tassel dwarf1 (td1) fail to restrict proliferation in inflorescence and floral meristems. Both male and female inflorescences are dramatically increased in size and sometimes show line or ring fasciation. SEM analysis reveals that female inflorescences show irregular initiation of spikelet pair meristems and asymmetric growth of spikelet pair and spikelet meristems. The female flower meristem often overproliferates, becoming indeterminate. The penetrance and expressivity of td1 varies greatly among different inbred backgrounds, with some inbreds completely masking the phenotype. Double mutant studies using td1 and other mutants affecting meristem function and flower development have been helpful in assigning regulatory relationships between td1 and other maize genes, as well as uncovering novel roles for td1 in various aspects of meristem development.

T4 The ramosa1 gene regulates inflorescence architecture and encodes a small zinc finger protein
Vollbrecht, Erik(1); Kopec, Charles(1); Goh, Lindee(1); Springer, Patricia(1); Martienssen, Rob(1) (1) Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724, USA

Much variation in plant form can be ascribed to regulated branching patterns that produce specific architectures. The tassel and ear have contrasting mature morphologies yet similar underlying architecture. In both, the primary inflorescence meristem is indeterminate, initiating a few hundred second-order meristems that sequentially produce determinate, higher order meristems. Differential activity of second order meristems determines the essential architectural difference between the ear and tassel. In the ear, all second order meristems function as determinate spikelet pair meristems, producing a spike architecture. In the tassel, second order meristems assume either of two opposing fates: a few initial ones become indeterminate branch meristems, and the rest become determinate spikelet pair meristems as in the ear. Thus the mature tassel is a panicle comprising a proximal zone of branches and a distal central spike. In ramosa1 (ra1) strong mutants, ears and tassels are fully branched panicles lacking a spike zone. Scanning electron microscope analysis of developing tassels indicates that functional ra1 imposes a determinacy switchpoint, to inhibit second-order meristems from becoming long branches in the central spike. ra1 also functions in the branch zone, but double mutant analysis indicates that in normal tassels a genetically separable pathway promotes branching at the base. Based on heritability data from sectored tassels carrying transposon-induced alleles, the ra1 gene product can function cell non-autonomously. We cloned ra1 by transposon tagging with Spm and have identified molecular lesions in 10 mutant alleles. The ra1 gene is expressed in developing tassels and ears but not in vegetative shoot tissues and encodes a 19kDa protein with a single TFIIIa-type zinc finger. We report ra1 expression data in normal and mutant inflorescences, and discuss ra1 function with respect to meristem determinacy and grass inflorescence architecture.

T5 The glossy early flowering gene is required for proper embryogenesis and plant development
Baker, Frank (1); Freeling, Michael (1) (1) University of California-Berkeley, Berkeley, CA 94720, USA

In the course of its development, the maize plant passes through two distinct vegetative phases, a juvenile phase and a subsequent adult phase. Previous characterization of recessive alleles in the glossy early flowering (gef) gene (also called early phase change (epc) by M. Sauer and R.S. Poethig in Poster 65 in the 42nd MGC) have focused on the abbreviation of the juvenile phase as evidenced in mutant plants. Analysis of a strong allele, gef-nl4, has revealed further developmental roles for gef. In addition to the abbreviation of the juvenile phase, gef-nl4 mutants display a perturbed embryo morphology in which a number of defects are apparent, including misalignment of the shoot and root poles, aberrant development of the root and shoot apices, and misgrowth of the vascular system. These defects become further evident during seedling development, manifesting as an absence of the mesocotyl, inhibited root growth, and high seedling mortality. Later in development abnormalities in the leaf occur. The leaves often display diffuse and/or multiple midribs and multiple leaf tips. The pleiotropic nature of the gef-nl4 mutant suggests that gef may play a general developmental role rather than a specific one. Any model proposing a function for this gene will need to account for its global effects. Such a model may incorporate the hormonal action of auxin and cytokinin, since gef-nl4 plants display phenotypes known to be associated with alterations in the levels or movement of these hormones.

SESSION 2 10:40-12:20 AM FRI

T6 Isolation of a novel autonomous class 2 (DNA) element with strong target site preference and MITE family members
Zhang, Xiaoyu(1); Jiang, Ning(1); Zhang, Qiang(3); Eggleston, William, B.(2); Feschotte , Cedric (1); Wessler, Susan, R.(1) (1) University of Georgia, Athen, GA 30502, USA; (2) Virginia Commonwealth University, Richmond, VA 23284, USA; (3) Monsanto Mystic Research, Mystic, CT 06355, USA

Miniature inverted-repeat transposable elements (MITEs) are non-autonomous DNA elements prevalent in grass genomes and also widespread in other plants, nematodes, insects and vertebrates. Despite their wide distribution and the evidence that some MITE families have recently spread through grass genomes, their origin and transposition mechanism have yet to be clearly elucidated. We have isolated and characterized a maize MITE called miniature PIF (mPIF). Family members are numerous (5,000 copies), well conserved in both nucleotide sequences (90%) and length (365 bp on average), have 14 bp terminal inverted repeats (TIRs), and show strong target site preference. Most significantly, mPIF elements share identical TIRs, similar subterminal sequences and identical target site preference with P instability factors (PIF), a DNA transposon family first identified by multiple mutagenic insertions into an identical site in intron 2 of the R locus. These similarities strongly suggest that mPIFs are non-autonomous members of the PIF family. We have explored the structure of the large PIF elements and employed the Transposon Display technique to identify and isolate the autonomous PIF element (PIFa) from a population segregating for PIF activity. Database searches revealed PIF homologs in rice, sorghum and Arobidopsis. The Arabidopsis homolog has also been suggested to be related to MITEs. Therefore, PIFa may represent the first identified autonomous element of a superfamily that is capable of giving rise to MITEs.

T7 Identification of the genes involved in the establishment and interpretation of DNA methylation patterns in maize. Springer, Nathan(1); Papa, Charles (1); Muszynski, Michael (2); Danilevskaya, Olga(2); Smith, Alan (1); Kerns, Sarah(1); Phillips, Ronald (3); Kaeppler, Heidi (1); Kaeppler, Shawn(1) (1) Dept of Agronomy, University of Wisconsin-Madison, 1575 Linden Dr, Madison, Wisconsin 53706, USA.; (2) Pioneer Hi-Bred International, Inc., 7300 N. W. 62ndAve, Johnston, Iowa 50131, USA.; (3) Department of Agronomy and Plant Genetics, University of Minnesota, 1991 Buford Cir, St. Paul, Minnesota 55108, USA.

A long-term goal of our lab has been to characterize genes involved in the establishment and interpretation of DNA methylation patterns in maize. Four distinct classes of DNA methyltransferase genes exist in maize, at least three of which have multiple family members. Three of the four classes are represented in both plant and animals, indicating an ancient evolutionary origin for these methyltransferases. The fourth class, the chromomethylases, is found only in plants. Our most extensive analysis to date has been on the chromomethylase Zmet2a. Plants homozygous for a Mu insertion allele of Zmet2a display 10-15% reduction in DNA methylation levels. Southern blot analysis of centromere and ribosomal sequences coupled with genomic bisulphite sequencing of the 180bp knob sequence indicates that the reductions in methylation are specific to CpXpG. CpG and asymmetric methylation are not affected in the mutant. Isolines for the zmet2-m1:Mu allele show no morphological differences. A transgenic event expressing a dominant negative inverted repeat construct for the Zmet2a gene corroborates the methylation defect observed in the zmet2-m1:Mu homozygotes. The observations that chromomethylases are found only in plants and are required for CpXpG methylation is consistent with the presence of high levels of CpXpG methylation in plants but not in animals. We have sequenced four maize genes containing a putative methyl-CpG-binding domain (MBD) corresponding to three of the eight classes of MBD genes found in Arabidopsis. The putative MBD proteins found in plants do not share any similarity to the mammalian proteins outside the conserved MBD. MBD proteins presumably interpret DNA methylation patterns established by DNA methyltransferase. While, phylogenetic analysis supports distinct lineages of DNA methyltransferases, these lineages are not paralleled in the evolution of MBD proteins. Plants and animals contain similar enzymes for the establishment of DNA methylation patterns but appear to utilize distinct classes of genes for the interpretation of these patterns.

T8 Sequences between ~90 and 103 kb upstream of the b1 transcription start site are required for paramutation and for high expression of the b1 gene
Stam, Maike(1); Belele, Christiane(1); Lennertz, Pascal(1); Chandler, Vicki L.(1) (1) University of Arizona, Tucson, Arizona 85721, USA

Paramutation is a mitotically and meiotically heritable change in the expression of one allele caused by another allele of the same gene. Paramutation has been described for a number of genes, one of which is b1, a regulatory gene of the anthocyanin pigmentation pathway in maize. At b1, the expression of an allele conferring dark purple pigment, B-I, is downregulated to the expression level of an allele conferring light purple pigment, B', in B'/B-I heterozygous plants. New B' alleles are like existing B' alleles, they paramutate naive B-I alleles with a 100% efficiency. B' is transcribed at a 10-20 fold lower rate than B-I (Patterson et al.,1993, Genetics 135:881). Both paramutation and the high expression of B-I require sequences upstream of the coding region (Patterson et al.,1995, Genetics 140:1389). To map the location of the sequences at the b1 locus required for 1) paramutation and 2) high expression, crosses were made to isolate recombination events upstream of the b1 coding region between B-P and B' or B-I. B-P is an allele insensitive to paramutation that confers little pigment in mature plants. Sixty-nine recombinant alleles have been isolated and genetically characterized for loss, gain or retention of the paramutation sequences. Identification of sequence polymorphisms between B'; B-I, and B-P allowed us to map recombination break points. These results show: 1) the paramutation sequences are located between ~90 and 103 kb upstream of the b1 transcription start site. 2) an enhancer influencing b1 expression levels is located between ~91 and 101 kb upstream of the b1 transcription start site. Thus, the paramutation sequences, and enhancer sequences, are located far upstream of the promoter proximal and coding region of b1, suggesting the involvement of long range communication between sequences 90 kbp upstream and the promoter proximal region. The nature of these sequences and a model for paramutation will be discussed.

T9 Post-transcriptional regulation and epigenetic control of the MuDR/Mu transposon family in maize
Rudenko, George N.(1); Walbot, Virginia(1) (1) Stanford University, Stanford, California 94305-5020, USA

Mu transposition, responsible for Mutator activity in maize, requires a transcriptionally active autonomous MuDR element. Regulation of the multi-copy, dispersed MuDR/Mu elements occurs at different levels. During plant development, transposition events are restricted to the last cell divisions and Mu excision frequencies are similar. The other main regulatory mechanism affecting the whole element family is a coordinate epigenetic silencing of MuDR elements followed by methylation of Mu termini. We report previously unsuspected and ubiquitous MuDR homologs that produce both RNA and protein. In active Mutator lines, MuDR transcript levels are proportional to the MuDR copy number, and expression of hMuDR is increased. In non-MuDR and epigenetically silenced MuDR lines, a subset of hMuDR elements remains transcriptionally competent. Surprisingly, despite a 10-20 fold difference in RNA levels in all tested Mutator and non-Mutator lines, immunodetection demonstrated nearly invariant levels of MuDR and homolog protein products. These results imply a strict, post-transcriptional control over protein production that might explain the uniform Mu excision frequency. Subsequent RNA analysis on genetically verified stocks allowed us to detect a correlation between initiation of transposon silencing and progressive retention of MuDR transcripts in nuclei, with highest levels observed in pollen (70-80%). Most of nuclear RNA is non-polyadenylated, which explains its failure to reach the cytoplasm. We propose that nuclear RNA triggers host defenses resulting in coordinate transposon methylation and transcriptional silencing. Furthermore, homolog transcripts in non-MuDR inbred and epigenetically silenced MuDR lines accumulate preferentially in nuclei (70-90%) and thus might account for the maintenance of the silenced state.

T10 Comparative Analysis of Regulation of Phlobaphene Biosynthesis in Maize & Sorghum
Chopra, Surinder(1); Olson, Terry(2); Peterson, Thomas(2) (1) Penn State University, University Park, PA 16802, USA; (2) Iowa State University, Ames, IA 50011, USA

The maize p1 gene regulates the biosynthesis of red phlobaphene pigments in different tissues including the kernel pericarp, cob and tassel glumes, husk and sheath. In sorghum, the candystripe pericarp phenotype specified by the Y1-cs has a striking similarity to the maize variegated pericarp phenotype specified by the P1-vv allele, which contains an Ac transposon insertion in the P1-rr. Our molecular analysis of the sorghum Y1-cs show that the y1 gene is closely related to the maize p1 gene, and that the pericarp variegation is caused by the activity of Candystripe1 transposable element (Chopra et al., 1999, PNAS 96, 15330). We isolated and sequenced a 30-kbp sorghum genomic contig, which includes the y1 gene and a homologous pseudogene termed y2. The y2 sequence is present in direct orientation, approximately 8.7 kbp 3' of y1 and the genomic arrangement of y1 and y2 resembles that of the maize p2/p1 complex (Zhang et al., 2000, Plant Cell 12, 2311). Sequence alignment of y1 with different alleles of p1 show that the divergent sequences observed in the 5' flanking regions are in sharp contrast to the fairly conserved coding sequences of these orthologous regulatory genes. The unique expression property conferred by the sorghum y1 in leaves has further lead to the possible identification of the determinants of differential regulation of 3-deoxyflavonoids biosynthesis in sorghum and maize. Our results give a striking indication of the remarkable plasticity of grass genomes, and the potential for altered gene expression via manipulation of transcription regulators.

SESSION 3 8:30-10:10 AM SAT

T11 The Etched 1 gene product of Zea mays contains a zinc ribbon-like domain and is homologous to the eucaryotic transcription elongation factor TFIIS

da Costa e Silva, Oswaldo(1,4); Garg, Preeti(1); Waßmann, Martina(1); Lorbiecke, René(1); Lauert, Patricia(1); Peters, Ulrike(1); Scanlon, Mike (2); Hsia, An-Ping(3); Schnable, Patrick S.(3); Wienand, Udo(1) (1) Institut für Allgemeine Botanik, AMP I, Universität Hamburg, Germany; (2) University Georgia, 3609 Plant Sciences, Athens GA 30602, USA; (3) Iowa State University, Ames, IA 50011, USA; (4) BASF Plant Science LLC, 26 Davis Dr, Research Triangle Park NC 27709, USA (present address)

Etched 1 (et1) is a pleiotropic mutation in maize affecting endosperm and seedling development. et1 kernels are fissured and cracked and et1 seedlings are virescent until approximately two weeks after germination. The etched 1 gene was identified from a Mutator-induced mutant allele using the AIMS (amplification of insertion mutagenized sites) technique. Several mutant alleles as well as the wild-type allele were cloned and analyzed molecularly. The etched 1 gene is about 3 kb in size and contains 4 exons. Expression analysis revealed transcripts, approximately 800 bp in size, in wild-type endosperm and leaves. The putatively encoded protein is 163 amino acids in length. It contains a zinc ribbon-like domain and shows homology to the eucaryotic transcription elongation factor TFIIS. The mRNA has been localized by in situ experiments in the outer cell layers of the endosperm. Organelle localization experiments revealed that the ETCHED 1 protein is transported into the stroma of chloroplasts. From the analyses of the etched 1 gene we conclude that the ETCHED 1 protein may be part of a transcription complex involved in plastid development.

T12 STARCH-DEBRANCHING ENZYME SUGARY1 IS KEY TO MAIZE KERNEL PHENOTYPES AND DOMESTICATION

Buckler, Edward S.(1,2); Rinehart-Whitt, Sherry(1); Le Thierry, Maud(3); Gaut, Brandon S.(3); Doebley, John F.(4); Goodman, Major M.(5) (1) USDA-ARS, Plant Science Research Unit.; (2) Dept. of Genetics, North Carolina State Univ., Raleigh, NC 27965, USA; (3) Dept. Ecology and Evolutionary Biology, U.CA Irvine, Irvine, CA 92697, USA; (4) Genetics Dept., 445 Henry Mall, Univ. of Wisconsin, Madison, WI 53706, USA; (5) Dept. of Crop Science, North Carolina State Univ., Raleigh, NC 27695, USA

The classic sweet corn phenotype is produced by a mutant at the Sugary1 (Su1) locus, which encodes for an isoamylase or starch-debranching enzyme. We surveyed Su1 nucleotide diversity in maize, and its wild relative, teosinte. A tryptophan to arginine mutation at conserved residue 578 was found in all sweet corn lines. This finding is corroborated by the molecular and biochemical work of Dinges et al. (2001 Plant Phys. 125: in press). We suggest this residue may be critical for substrate specificity in isoamylases. Our survey also discovered that popcorns have an allele similar to sweet corns except for the W578R mutation, while most flint and dent maize have a highly differentiated alleles. Overall, maize had very low diversity when compared to teosinte indicating that this gene was probably a key element of maize domestication. Maize lines with sweet and pop phenotypes appear to have alleles very similar to those found in Zea mays ssp. parviglumis, while most dent and flint maize have a haplotype that is infrequent in maize's wild relatives. Su1 appears to be a key gene in maize domestication, and breeding with some of the identified Su1 alleles may produce maize with modified starch and protein levels.

T13 Allelic variation at the sugary1 locus provides insight into the molecular mechanisms of starch biosynthesis

Dinges, Jason(1); Colleoni, Christophe(1); Lightner, Jon(2); Pearlstein, Richard(2); Myers, Alan(1); James, Martha(1) (1) Iowa State University, Ames, Iowa 50011, USA; (2) DuPont Agricultural Products, Newark, Delaware 19714, USA

Starch production in all plants examined is altered by mutations of isoamylase-type starch debranching enzymes (DBE), although how these proteins affect glucan polymer assembly is not understood. Various allelic mutations in the maize sugary1 (su1) gene, which codes for an isoamylase-type DBE, condition distinct kernel phenotypes. This study characterized several genetic and transposon-induced alleles of su1, comparing gene expression, kernel glucan structures and composition, and effects on starch metabolizing enzymes. Genetic mutations examined included su1-Ref, which was shown to result from nucleotide changes that lead to two amino acid substitutions but do not alter mRNA levels, su1-R4582::Mu1, which is a null allele, and su1-st, which results from insertion of a novel transposon-like sequence that causes alternative pre-mRNA splicing. This leads to production of three su1-st transcripts, one that is nonfunctional, and two that code for slight amounts of modified SU1 polypeptides. Although su1-st kernels accumulate significantly less phytoglycogen and sucrose, all three alleles severely reduce or eliminate isoamylase-type DBE activity, as measured in native PAGE activity gels. These zymograms also revealed several allele-specific effects on other starch metabolizing enzymes, including the unique loss of branching enzyme IIa activity in su1-st kernels. Analysis of the chain lengths in residual amylopectin showed significantly more short chains and fewer medium and long chains in both su1-Ref and su1-R4582::Mu1 kernels, with more modest effects conditioned by su1-st. Similar analysis of antisense or gene-silenced transgenic Su1 plants showed similar changes in the amylopectin structure. Conversely, transgenic plants in which Su1 was over-expressed had amylopectin with fewer short chains and more medium to long chains. Together, these results suggest that SU1 directly participates in amylopectin biosynthesis, but that its catalytic activity is the not the sole determinant of Su1 function; rather, specific interactions between SU1 and other components of the starch biosynthetic system are required.

T14 Maize phytoene desaturase (PDS) and zeta-carotene desaturase (ZDS) produce poly-Z-lycopene: Implications for genetic manipulation of carotenogenesis in maize and rice

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Enzymes in the carotenoid biosynthetic pathway may be sensitive to the geometric isomer states of their substrates; progression of substrates through the pathway may depend on obligate isomerase activities. Such isomerases may also be rate-controlling. Metabolic engineering for vitamin A content ex situ, requires knowledge of ancillary factors affecting carotenogenesis. Functional analyses of the concerted action of maize phytoene desaturase (PDS) and zeta-carotene desaturase (ZDS) by combined HPLC separation and PDA analyses of geometric isomers of carotenoids accumulating in E. coli. have shown maize PDS and ZDS to mediate a poly-Z-desaturation pathway to 7,9,7',9'-Z-lycopene (poly-cis-lycopene or prolycopene). The existence of a poly-Z-desaturation pathway for maize PDS and ZDS is discussed in relation to two divergent interpretations. For each interpretation implications for genetic manipulation and further study of maize and rice carotenogenesis are given.

T15 ADP-GLUCOSE PYROPHOSPHORYLASE ACTIVITY FROM MAIZE-POTATO MOSAICS
Cross, Joanna MF.(1); Greene, Thomas W.(3); Shaw, Janine R.(1); Clancy, Maureen(1); Okita, Thomas W.(2); Schmidt, Robert R.(1); Hannah, L. Curtis(1) (1) University of Florida, Gainesville 32611, Florida; (2) Washington State University, Pullman 99164, Washington; (3) Dow AgroSciences, Indianapolis 46268, Indiana

The allosteric enzyme, adenosine diphosphate glucose pyrophosphorylase (AGP) catalyses the synthesis of ADP-glucose, a rate limiting step in starch synthesis. Plant AGPs are heterotetramers activated by 3PGA and inhibited by Pi. The objective of these studies is to identify regions in the subunits important in regulation and activity. We exploit an E.coli expression system and mosaic AGPs composed of potato tuber and maize endosperm subunit fragments to unravel this question. While potato and maize subunits have long been separated by speciation and evolution, the potato tuber and maize endosperm subunits are sufficiently similar to form active mosaic enzymes. Since potato tuber and maize endosperm AGPs exhibit different regulatory properties, comparing the kinetic properties of the mosaics to those of the maize endosperm and potato tuber AGPs has enabled us to identify regions important in regulation. So far, we have identified a region in the N terminus of the small subunit that is essential in 3PGA regulation, and another region in the C-terminus of the small subunit important for subunit interaction or activity. In contrast to conclusions drawn by other investigators, the data presented here conclusively show that the small subunit plays a pivotal role in the regulation of AGP.

SESSION 4 10:40 AM-12:20 PM SAT

T16 Mitochondrial RNA Polymerase: Reverse Genetics and Biochemistry
Boisson, Murielle(1); Caoile, Angel(1); Stern, David(1); Chang, Ching-Chun(1); Lerbs-Mache, Silva(2); Meeley, Robert(3) (1) Boyce Thompson Inst., Cornell Univ., Ithaca NY 14853; (2) Universite Josef-Fourier, Grenoble, France; (3) Pioneer Hi-Bred International

We have previously characterized two nuclear genes encoding related RNA polymerases of the T7 family (1). GFP fusions showed that one product, RpoTp, was directed to chloroplasts whereas the other RpoTm, was directed to mitochondria. We have taken a dual approach to understanding the function of RpoTm. First, we have characterized a Mu insertion allele obtained through the TUSC collection. Even after numerous outcrossings, plants carrying mutant alleles of rpoTm segregate variable proportions of seedling or embryo lethals, whereas siblings carrying two wild-type alleles do not. The proportion of affected individuals varies among progeny of selfed heterozygotes, and the genotype of rpoTm does not correlate with the phenotype, suggesting the effect is exerted in the prior generation. Occasional homozygotes survive the seedling stage, and the relevant data will be presented. A second approach is biochemical: RpoTm and RpoTp have been expressed in bacterial cells and have strong transcription activity. We will report our progress in identifying potential specificity factors for these nonspecific core polymerases. (1) Chang, C, Sheen, J, Bligny, M, Niwa, Y, Lerbs-Mache, S and Stern, DB (1999). Functional analysis of two maize nuclear genes encoding T7-like RNA polymerases. Plant Cell 11:911-926.

T17 Programmed cell death in S male-sterile maize

Chase, Christine (1); Wen, Lan-ying(1); Gallagher, Larbi(1); Jamoom, Eric(1); Ortega, Victor(1) (1) University of Florida, Gainesville, Florida 32611, USA

Cytoplasmic male sterility (CMS), the maternally inherited failure to produce functional pollen, results from the expression of novel, chimeric genes in the mitochondria. The chimeric gene sequences differ among the different CMS systems. We do not currently know how expression of these genes results in male sterility, or why male sterility is the only phenotypic consequence of CMS gene expression. Based upon the role of mitochondria in signaling programmed cell death (PCD) in vertebrates (Green and Reed, Science 281:1309), we propose that expression of CMS genes in male reproductive tissue initiates a PCD signal cascade, resulting in male sterility. In CMS-S maize, reproductive failure occurs in developing male gametophytes. These can be purified in abundance for investigation of the molecular and cellular events that accompany pollen abortion (Wen and Chase, Sex Plant Reprod 11:323). The morphology of aborting CMS-S pollen (Lee et al. Am J Bot 67:237) bears striking similarity to that of animal cells undergoing PCD. We examined CMS-S pollen for molecular features of PCD. Mitochondria purified from collapsed CMS-S pollen were depleted of cytochrome c, an early event in the signal transduction cascade leading to PCD in vertebrates (Bossy-Wetzel et al. EMBO J 17:37). Collapsed CMS-S pollen contained degraded RNA, degraded DNA and novel proteases, all features associated with the execution phase of PCD. Transcripts of two chimeric mitochondrial open reading frames, orf355 and orf77, are correlated with CMS-S (Zabala et al. Genetics 147:847). Sequence analysis of CMS-S pollen mitochondrial cDNA clones revealed no effects of orf355/orf77 transcripts on the RNA editing of normal mitochondrial gene transcripts. We therefore propose that protein-level expression of orf355 and/or orf77 alters mitochondrial structure or function to signal PCD. Chimeric mitochondrial gene products potentially signal PCD in other CMS systems, and we suggest that natural selection has acted to eliminate these signals in vegetative and female reproductive tissues.

T18 THE CELL DEATH SUPPRESSING FUNCTION OF *lls1*, (*lethal leaf-spot 1*) INVOLVES PROTECTING CHLOROPLAST INTEGRITY.

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The integrity of the mitochondrion and reactive oxygen species (ROS) production play key roles in regulating programmed cell death (PCD) in animals. A similar role for mitochondrial regulation of PCD in plants has not yet been established. Study of the *lls1* mutation in maize and its ortholog (*acd-1*) in Arabidopsis suggest that control of chloroplast integrity may play a key role in regulating plant cell death. The *lls1* lesion mimic gene is recognized by its recessive mutations, which exhibit the phenotype of propagative lesions in the absence of a pathogen infection. The lesions are formed on leaf tissue in a developmental gradient that approximately parallels chloroplast maturation. In addition light is required for the progression of lesion formation, which can be triggered by wounding or infection. More significantly lesion development is dramatically reduced in a photosynthetically defective oil-yellow 700/lethal leaf-spot double mutant (*Oy700/+, lls1/lls1*). Lesions do not form in the albino sectors of an iojap/lethal leaf-spot double mutant (*ij1/ij1 lls1/lls1*). Electron microscope studies suggest that ultrastructural changes occur in the chloroplasts of wounded *lls1* plants prior to any other cellular changes. The molecular function of the LLS1 protein is not yet known but *lls1* expression in green leaves increases almost one hundred fold following physical wounding. Sequence analysis of the cloned lls1 gene predicts a chloroplast transit peptide cleavage site and the presence of two non-haem iron-binding motifs in the LLS1 protein. These iron binding motifs appear to have been recruited during evolution for a variety of functions in bacteria and plants. It is clear that LLS1 provides an important protection against stress-induced cell death in higher plants. From our observations we hypothesize that LLS1 protects the plant from a chloroplast derived intermediate which becomes a free radical due to excess light or by oxidative stress during wounding. In contrast to mitochondrial regulation of PCD in animals it is possible that withdrawal of a chloroplast protective function such as this could regulate the initiation of PCD events in plants.

T19 Genetic mapping of gray leaf spot (GLS) resistance genes in maize
Lehmensiek, Anke(1); Esterhuizen, Adriaan, M.(2); van Staden, Derick(1); Nelson, Pip,W.(2); Retief, Andries, E. (1) (1) University of Stellenbosch, Stellenbosch 7600, RSA; (2) Sensako,Brits 0250, RSA

Bulked segregant analysis was used to identify amplified fragment length polymorphism markers (AFLPs) linked to quantitative trait loci (QTLs) involved in the resistance to gray leaf spot (GLS) in maize. By using 10 AFLP primer combinations 11 polymorphic markers were identified and converted to sequence-specific PCR markers. Five of the 11 converted AFLPs were linked to three GLS resistance QTLs. The markers were mapped to the maize chromosomes 1, 3 and 5 using existing linkage maps of two commercially available recombinant inbred line populations. Converted restriction fragment length polymorphism markers and microsatellite markers were used to obtain a more precise localization for the detected QTLs. The QTL on chromosomes 1 was localized in bin 1.05/06 and had a LOD score of 21. A variance of 37% was explained by the QTL. Two peaks were visible on chromosome 5, one was localized in bin 5.03/04 and the other in bin 5.05/06. Both peaks had a LOD score of 5 and 11% of the variance was explained by the QTLs. A variance of 8-10% was explained by the QTL on chromosome 3 (bin 3.04). The consistency of the QTLs was tested across two F2 populations planted in consecutive years.

T20 An herbivore elicitor activates the gene for indole emission in maize
Frey, Monika (1); Tumlinson, Jim(2); Gierl, Alfons(1) (1) Lehrstuhl f. Genetik, Technische Universitaet Muenchen; (2) USDA, ARS, CMAVE, Gainesville

Maize and a variety of other plant species release volatile compounds in response to herbivore attack that serve as chemical cues to signal natural enemies of the feeding herbivore. N-(17-hydroxylinolenoyl)- L-glutamine is an elicitor component that has been isolated and chemically characterized from the salvia of the herbivore pest beet armyworm. This fatty acid derivative, referred to as volicitin, triggers the synthesis and release of volatile components, including terpenoids and indole in corn. A gene that encodes an indole- 3-glycerol phosphate lyase (IGL) catalyses the formation of free indole and is selectively activated by volicitin. IGL,s enzymatic properties are similar to BX1, a maize enzyme that serves as the entry point to the secondary defense metabolites DIBOA and DIMBOA. Gene sequence analysis indicates that Igl and Bx1 are evolutionary related to the tryptophan synthase alpha subunit.

SESSION 5 9:00-10:40 AM SUN

T21 Cloning of narrow sheath2, a duplicate factor gene required for recruitment of a lateral domain in the maize leaf.

Scanlon, Michael J.(1); Nicholson , Benjamin M. (1); Ji, Jaibing (1) (1) Botany Department, University of Georgia, Athens, GA, 30602, USA

The duplicate-factor genes narrow sheath1 (ns1) and narrow sheath2 (ns2) are required for the development of a lateral domain in maize phytomers that includes the margins of the stem, leaf sheath and lower blade. Immunohistochemical analyses of KNOX protein accumulation, and fate-mapping of maize meristems indicate that plants homozygous for ns1 and ns2 fail to recruit meristematic founder cells that normally contribute to this lateral domain of the maize phytomer. Clonal analyses support a model in which NARROW SHEATH1 functions to recruit maize stem/leaf founder cells from two marginal foci in the maize meristem. For accessibility in molecular cloning, a strategy was designed to identify putative Mutator transposon-tagged alleles of each of the narrow sheath duplicate genes; these new mutant alleles are designated ns2-Mu77 and ns1-Mu78. A 4.8 kb MuDR-homologous, BamHI restriction fragment was found to co-segregate with the ns2-Mu77 mutation; this ns2-linked fragment was cloned into phage lambda. Several lines of molecular and genetic evidence are presented to indicate that ns2 has been cloned.

T22 Beta-keto acyl reductase activity is essential for maize development

Dietrich, Charles R(1); Mahapatabandige, Perera A(1); Meeley, Robert B (2); Nikolau, Basil J(1); Schnable, Patrick S(1) (1) Iowa State University; (2) Pioneer Hi-bred Intl.

Mutations in the *glossy8* (*gl8)* gene condition a "glossy" seedling phenotype resulting from a reduced cuticular wax load on the leaves. The *gl8* gene encodes the beta-keto acyl reductase component of the fatty acid elongase complex which generates the very long chain (>30C) fatty acid precursors used in cuticular wax biosynthesis (Xu *et al.* 1997; Xu *et al*. in prep.). The maize genome contains a nearly identical (97%) paralog of *gl8* (now termed *gl8a*)*, gl8b.* A *Mu* transposon insertion in this paralog (*gl8b*) does not cause any obvious mutant phenotype. Analysis of RNA accumulation reveals that both genes are expressed throughout development but that the *gl8a* gene is predominantly expressed in seedling leaves, silk and developing ears. In contrast, *gl8b* is predominantly expressed in seedling roots and developing ears. Homozygous mutations at either locus alone produce plants with essentially normal morphologies. In contrast, double mutants arrest early in development. Kernels with the double mutant genotype typically do not germinate. The approximately 20% that do germinate produce normal primary and seminal roots but little or no shoot growth. The high level of expression of both *gl8a* and *gl8b* in developing ears and the lethality conditioned by the double mutant suggest that the very long chain fatty acids generated from the GL8-containing fatty acid elongase complex are required for normal embryo development.

T23 Discovery and Characterization of Maize Alternatively Spliced Genes by EST Analysis
Lal, Shailesh(1); Wang, Bing-Bing(1); Bunner, Anne (1); Valand, Renate(1); Brendel, Volker(1) (1) Iowa StateUniversity, Ames, Iowa 50011-3260

Alternative splicing allows generation of multiple transcripts from a single gene by selection of different combinations of splice sites in the given pre-mRNA. Regulation of gene expression via alternative splicing is very prevalent in vertebrates. The extent of this process in plants remain undetermined. However, a growing number of examples suggests that this phenomenon is far more prevalent in plants than initially anticipated. Large collections of plant ESTs deposited in the public databases provide an important resource to study this process from a genome wide perspective. We have searched 73,128 maize ESTs available at ZmDB (http://.zmdb.iastate.edu) and have identified 362 genes as candidates for alternative spliced expression. Of these, 151 ESTs does not bear significant similarity to protein sequences in public databases. To determine the efficacy of our computer search, we have randomly selected 40 annotated genes and performed RT-PCR using primers that were complementary to the sequence flanking the tentative alternatively spliced introns. We also used the same set of primers in PCR reactions to amplify the cognate genomic sequences. The results of these studies and the possible biological relevance of the translation product of the alternatively spliced transcripts are presented.

T24 Isolation and characterization of sperm-expressed genes in *Zea mays*
Engel, Michele L.(1); Chaboud, Annie(2,3); Dumas, Christian (2); McCormick, Sheila (1) (1) Plant Gene Expression Center, USDA/ARS-UC-Berkeley, 800 Buchanan St., Albany, California 94710, USA; (2) Laboratory of Plant Reproduction and Development, UMR 5667, Ecole Normale Supérieure de Lyon, CNRS-INRA-ENS, Lyon-UCB Lyon I, 69364 Lyon Cedex 07, France; (3) Flow Cytometry Facility, Ecole Normale Supérieure de Lyon, Lyon, Cedex 07, France

Although a great deal of information is available about gamete recognition in animals, molecules that mediate gamete (egg-sperm) recognition and signaling are unknown in plants. Plant sperm are plasma membrane-bound cells that are unusual in that they are enclosed within the cytoplasm of the vegetative cell of the pollen grain. Until recently, fractions enriched for sperm cells were still highly contaminated with vegetative cell cytoplasm, and thus information about gene expression specific to male gametes was essentially lacking. Using 1 million FACS-sorted sperm, we obtained 1.8 micrograms of total RNA. We constructed a cDNA library with the SMART cDNA cloning kit from Clontech. Our cDNA sizes range from 300 bp to 4 kb, with most in the 800-1300 bp range. A portion of the cDNA was ligated into lambda arms and packaged: the titer of the unamplified library was 2.3×10^6 pfu. The amplified library has a titer of 1×10^9 pfu/ml. We selected 116 random clones from the unamplified library; sequence analysis of these suggests that the library clones represent diverse sequences. While many of the sequences have been previously identified, some are not present in existing EST databases, and for others the best or only matches are to maize ESTs from anther, tassel, or glume cDNA libraries. We will sequence ~1000 clones in order to get a general picture of gene expression in sperm, and will determine the expression pattern for selected clones via RT-PCR and whole mount in situ hybridization. Our primary interest is in proteins that are involved in egg-sperm interactions. Because such proteins are likely to be plasma membrane-bound or secreted, we are initiating a screen to enrich for cDNAs encoding N-terminal signal sequences, using a yeast mutant that can't grow on sucrose unless invertase is secreted.

T25 Isolation of maize genes with an imprinted pattern of expression.
Gutierrez-Marcos, Jose F.(1); O'Shea, Suzanne(1); Vanderpump, Sarah(2); Greenland, Andy (2); Dickinson, Hugh(1) (1) Dept. Plant Sciences, Oxford University, Oxford, OX1 3RB, UK.; (2) Syngenta, Jealotts Hill Research Station, Bracknell, Berks, RG12 6EY.

Endosperm is the product of a double fertilization process that occurs in most angiosperms. The egg and one of two sperm cells fuse to produce the embryo, while the two polar nuclei of the megagametophyte fuse with the other sperm to generate the triploid primary endosperm nucleus. Development of the endosperm in most angiosperms is required for the viability of the embryo and it has been demonstrated that the genomic ratio 2:1 (2 maternal:1 paternal) can be crucial for the successful development of this tissue. Any divergence from this ratio normally results in abortion of the endosperm. The molecular and cellular consequences for parental genomic interactions are thus highly important. A molecular mechanism must exist to sense the balance between the contribution of both parental genes, in that when an incorrect balance is detected, development is arrested. There is accumulating evidence that this molecular mechanism involves a system of gametic imprinting. Gametic imprinting is a unique form of epigenetic inheritance by which expression of certain genes, from generation to generation, is governed by their parental origin. Perhaps the most striking feature of imprinted genes is that active and inactive parental alleles coexist within individual cells. In plants, current evidence suggest that the embryo is less susceptible to the effects of gametic imprinting, than the endosperm. To date, only four different genes have been found to be imprinted in the plant endosperm, and in every case, maternally-inherited alleles are undermethylated and highly expressed. In order to explore the role that gametic imprinting plays in endosperm development, and to reveal the molecular mechanisms involved, we have identified and characterised two maize gene classes that present opposite parent of origin patterns of expression in the maize endosperm.

RICE GENOMICS WORKSHOP
7:00-10:00 PM FRIDAY MAR 17

W1 Rice genome sequencing efforts in IRGSP and RGP

Takuji Sasaki, Rice Genome Research Program, National Institute of Agrobiological Resources / STAFF-Institute, 1-2, Kannondai 2-chome, Tsukuba, Ibaraki 305-8602, Japan

Rice is one of the important cereal crops supplied for daily meal of about a half of the world population. In 1998, the ambitious project, rice whole genome sequencing was launched. Considering the importance of rice as main staple and reference for other cereal crops, the efforts of sequencing rice genome have been shared by many countries. IRGSP (International Rice Genome Sequencing Project) was organized as a collaboration to complete sequencing under guidelines regarding sequencing strategy, sequence accuracy and data releasing policy. Sequencing is based on shotgun sequencing of PAC/BAC clones aligned in contigs along each chromosome. RGP is constructing a contig (sequence-ready physical map) by screening PAC/BAC libraries with sequence information of many DNA markers generated by its own efforts. Additionally the large volume of STC (sequence-tagged connector) and fingerprints offered by Monsanto help to accelerate this job. RGP will finish physical mapping and sequencing of almost all the region of chromosome 1 by the end of March 2001. The physical length of this chromosome is about 42Mb and a longer genetic distance than average is observed. Under the IRGSP collaboration, the remaining chromosomes will be sequenced with more than a phase 2 level quality within a year and be completed in the near future. The huge amount of digitized data must be decoded by both biological and computational methods to clarify the mechanism of inheritance in rice plant and cereal crops.

W2 The CCW Rice Genome Sequencing Consortium: Sequencing the Short Arms of Chromosomes 10 and 3

Rod Wing, Cari Soderlund, Yeisoo Yu, Long Mao, Rob Martienssen, Rick Wilson and W. Richard McCombie. **C**lemson University Genomics Institute, Cold Spring Harbor Laboratory, **W**ashington University Genome Sequencing Center

The objectives of the CCW Rice Genome Sequencing Consortium, funded in October 1999[1], are to sequence and annotate the short arms of rice chromosomes 10 and 3. As a prelude rice genome sequencing, CUGI has focused on the development of a sequence tagged connector (STC)/BAC fingerprint framework to facilitate the International Rice Genome Sequencing Project (IRGSP). The framework consists of 4 elements: 1) two deep-coverage large insert BAC libraries (*Hind*III and *Eco*RI)[2]; 2) a STC database[3]; 3) a Fingerprint database[3] and 4) a Genome Anchoring database. Combined, the BAC libraries cover approximately 26 genome equivalents. The STC-DB is composed of DNA sequence derived from the ends of the DNA inserts in the *Hind*III and *Eco*RI BAC libraries and comprise over 40 Mb of high-quality rice genomic sequence deposited in Genbank. The Fingerprint DB contains, at a cut off of 1 X 10^{-12}, 63,233 clones assembled into 1038 contigs and 2927 singletons. The Genome Anchoring DB currently contains 603 markers (RFLPs and Overgos derived from STCs) have been placed on the physical map thereby anchoring 472 of the 1038 BAC contigs (45%). We estimate the 472 contigs cover about 215-234 Mb (50-54%) of the rice genome. Data will be presented describing the Framework Project, CCW's sequencing progress to date, and early applications of our sequencing efforts to better understand and sequence the rice genome.
Funded by: [1] USDA-CSREES/NSF/DOE Rice Genome Sequencing Program, [2] Rockefeller Foundation, [3] Novartis

Where the Monsanto rice sequences fit in.
Gerard Barry, Monsanto

Impact of Syngenta's recent announcement on rice
Steven Briggs, Syngenta

The Transposable Elements of rice.
Sue Wessler, University of Geogia

W3 DeleteageneTM: The Ultimate Knockout Technology for Rice Functional Genomics
Xin Li1, Yujuan Song1, Karen Century1, David McElroy2 and Yuelin Zhang1
institutions
1Maxygen Inc., Davis, CA, 95616, USA; 2Maxygen Inc., Redwood City, CA 94062 USA

Rice genome sequence information is accumulating in both public and private databases such that the potential for large-scale reverse genetics is fast becoming a reality in this model cereal. However, rice has a genome size that is at least three times larger than Arabidopsis and it is less readily amenable to transformation than this model dicot species. Therefore, it will be relatively difficult to saturate the rice genome using the kinds of T-DNA or transposon-based gene knockout systems that have been so successfully exploited in Arabidopsis. With this in mind we have developed a new reverse genetics methodology (DeleteageneTM) that can be used for efficient large-scale gene knockout in rice. By using a preferential PCR method to screen a fast neutron-mutagenized population comprised of 51,840 mutant families we have demonstrated that we can obtain deletion mutants for over 80 % of targeted loci in Arabidopsis. At the same time, we have developed an initial rice fast neutron population of about 25,000 mutant families. The high frequency of M2 generation albino plants indicates that fast neutron mutagenesis is highly effective in rice. Using a similar approach as that used in Arabidopsis, we have been able to identify deletion mutants in the rice fast neutron population. It is anticipated that, by expanding the size of the rice fast neutron population, we can saturate the whole rice genome with easily detectable gene deletions. DeleteageneTM does not require transformation, it can be applied to a wide variety of crop species, it is effective at knocking out small genes and it can be used to delete tandem duplicated genes. We see three main applications for DeleteageneTM reverse genetics technology in rice and other crop plants: plant gene function analysis; the generation of non-GMO plant gene deletion products and the identification and validation of agrochemical targets in crop plants.

W4 THE TIGR RICE GENOME PROJECT: SEQUENCE AND TOOLS FOR PLANT BIOLOGISTs

C. Robin Buell, The Institute for Genomic Research, 9712 Medical Center Dr, Rockville, MD 20850.

TIGR is participating in the International Rice Genome Sequencing Project and has been assigned chromosomes 3 and 10. We are collaborating with Clemson University/Cold Spring Harbor Laboratory/Washington University and the Plant Genome Initiative at Rutgers to complete these chromosomes. We currently have ~15 Mb of rice genomic DNA in our high throughput sequencing pipeline (http://www.tigr.org/tdb/rice). All sequence is released to Genbank/DDBJ/EMBL to either the High Throughput Sequence (HTGS) or the PLANT division. A total of ~11.5 Mb has been deposited in Genbank. We have in production all clones for our allocation on chromosome 10 (lower arm) with the exception of two small gaps. All completed BACs are annotated for genes and this information can be accessed through Genbank and the TIGR web site (http://www.tigr.org/tdb/rice). We have begun providing automated annotation of our unfinished rice BACs, for which this annotation is available on the TIGR web site. We have extended our bioinformatic analyses of rice and have identified putative orthologues of rice using the TIGR Orthologous Gene Alignments (http://www.tigr.org/tdb/toga/toga.shtml). We also have performed global alignments of rice genome sequences with all available plant transcripts to further identify candidate orthologous genes. As with all other TIGR Rice information, these data can be accessed on the TIGR Rice web site at www.tigr.org/tdb/rice.

W5 Naturally occurring variations as a new resource for functional genomics in rice

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Recent progress in rice genome analysis has made it possible to analyze the naturally occurring allelic variation underlying complex traits. Using heading date as a model for complex traits, we detected and characterized quantitative trait loci (QTLs), and identified genes at QTLs at the molecular level. QTLs for heading date were mapped by using several types of progeny derived from a cross between varieties Nipponbare and Kasalath. Nine QTLs were mapped precisely as single Mendelian factors by the use of advanced backcross progenies. Nearly isogenic lines (NILs) of QTLs were also developed by marker-assisted selection and were used to identify the function of each detected QTL. Combining 2 QTLs into the genetic background of Nipponbare allowed us to investigate epistatic gene interactions among QTLs. We analyzed a large segregating population by genetic and physical mapping to narrow down candidate genomic regions for target QTLs. These analyses revealed 10- to 50-kb regions as candidate regions for the target genes. Sequencing and expression analyses were used to identify the candidate genes. We identified genes or the most probable candidates for the photoperiod sensitivity loci Hd1, Hd3a and Hd6. Function of these candidate genes was verified by a genetic complementation test using Agrobacterium-mediated transformation. The results show that analysis of naturally occurring allelic variations would complement mutational analysis in elucidating the genetic control of complex traits such as heading date in rice. We also developed several permanent mapping populations, including recombinant inbred lines, doubled haploid lines, and chromosomal segment substitution lines from an indica / japonica cross. These plant materials will facilitate the genetic and molecular analysis of complex traits in rice. Currently, we are employing this strategy to clarify the genetic and molecular bases of several complex traits, including seed dormancy, shattering habit, and internode elongation and pollen sterility. Naturally occurring allelic variation could be a new resource for the functional analysis of rice genes.

3:30-6:00 PM SATURDAY MAR 17 MAIZE GENOMICS & FUTURES DIRECTIONS WORKSHOP

W6 High resolution mapping of a 550 kb YAC contig spanning the *rp1* disease resistance locus
Davière, Jean-Michel(1); O'Sullivan, Donal M.(1); Edwards, Keith J.(1,2) (1) IACR-Long Ashton Research Station, Department of Agricultural Sciences, University of Bristol, Long Ashton, Bristol BS41 9AF, United Kingdom ; (2) Department of Biological Sciences, University of Bristol, Woodland Rd, Bristol BS8 1UG, United Kingdom

Contiguous physical coverage of all seven members of the resistance gene family clustered at the complex *rp1* locus in the LH82 background has been obtained through the high resolution mapping of 13 YAC clones isolated using locus-specific probes in a 4-step chromosome walk. A minimum tiling path of 5 YAC clones spans approximately 550kb around the locus. Subclones carrying intact copies of each of the seven *rp1* homologues have been isolated, and their sequence is being determined. Of the *rp1*-related ORFs, several harbour deletions and insertions of various lengths. We will present first indications as to the structural organisation and gene content of this highly recombinogenic locus derived from sample sequencing of subclone libraries.

W7 CONSTRUCTION OF A PHYSICAL MAP FOR POSITIONAL CLONING IN CORN: ESTs, SNPs AND BACs
Faller, Marianna L(1); Fengler, Kevin A (1); Dam, Thao(1); Dolan, Maureen (1); Tingey, Scott V(1); Morgante, Michele (1) (1) DuPont Genomics, Newark, DE 19714-6104

Many relevant traits are controlled by unknown genes that can be genetically mapped but not easily identified. If their function is unknown, traditional gene isolation strategies cannot be used. A positional cloning approach is then the only option left. The availability of a physical map that is tightly linked to the genetic map and the mapping onto it of ESTs possibly representing all corn genes could make positional cloning an almost trivial exercise, once the genetic mapping is performed. Given that the gene density per cM is nearly the same between corn and Arabidopsis, positional cloning would not be any more difficult in corn from the point of view of gene isolation, once the position information for all genes is available. We have completed the construction of a whole-genome physical contig map of maize using fluorescent fingerprinting of a 10X corn BAC library (average insert size 155 kb). We will describe here the strategy adopted and the results achieved using a novel fingerprinting technology with 4 different fluorescent dyes in a single tube reaction and type IIS restriction enzymes. The process for high-throughput fingerprinting will be described and an update on the status of the map construction will be given. Our initial results of the mapping of tens of thousands of ESTs onto BACs will also be presented. We will discuss ways to efficiently utilise the physical map and how to link it to the genetic map.

W8 Using methylation filtered maize clones to identify new Mutator insertions in the Maize Targeted Mutagenesis

Rabinowicz, Pablo(1); Vollbrecht, Erik(1); May, Bruce(1); Yordan, Cristina(1); Dedhia, Neilay(1); Stein, Lincoln (1); McCombie, W.Richard(1); Martienssen, Robert(1) (1) Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 11724, USA

The genomes of most of the economically important grasses like maize are very large. This makes it unaffordable to approach genome-wide gene discovery by full genomic sequencing. It has been proposed that, regardless of the large range of genome sizes, all diploid higher plant genomes share essentially the same set of ~25,000 genes, called the "gene space". Variability in the content of highly repetitive DNA accounts for the genome size differences. It has been shown that most of these repetitive DNA is composed mainly by retrotransposable elements which are heavily methylated. On the other hand, genes are usually located in non-methylated regions of the genome. We have shown that repeat sequences in maize, because of their sensitivity to bacterial restriction-modification systems, can be largely excluded from genomic shotgun libraries by the selection of an appropriate host strain. In contrast, unmethylated genic regions are preserved in these genetically filtered libraries if the insert size is less than the average size of genes. In order to test the hypothesis that the gene space can be selectively isolated from any complex plant genome we are applying this technology to other plants. In addition to this genome-wide gene discovery approach we generated a large population of maize plants containing new insertions of transposon Mu to systematically determine gene function. We established a Center for Maize Targeted Mutagenesis (MTM; http:mtm.cshl.org) which uses this population to identify individual genes as a service to the community. We are currently combining these and DNA microarray technologies to disrupt and identify maize genes in a comprehensive way. We generated microarrays of filtered maize clones and hybridized them against representations of pools of Mu insertion sites. Using this approach, we identified several insertions in new maize genes.

W9 A Novel Method of Insertion Detection in Maize

Zhang, Qiang(1); Chomet, Paul(1); Kumpf, Steve (1); Hallowell, Shawn (1); West, Natalie (1); Lebejko, Sara(1); McNamara, Scott(1); Dellaporta, Steve(2) (1) Monsanto, Mystic, CT, 06355, US; (2) Yale University, New Haven, CT, 06520

Insertions alleles or loss of function alleles can assist in gene functional analysis by defining genetic, physiological, or biochemical effects. Transposon-induced mutations are isolated for known gene sequences by the general strategy known as „site-selected‰ mutagenesis. The method relies on the ability to use the PCR to amplify a collection of specific junction fragments between an inserted transposable element and a known target gene sequence from large pools of plants carrying randomly inserted elements. Seed segregating for the insertion allele can be identified and used in further studies to characterize the gene of interest. This method can be tedious, expensive, and time consuming due to the dependency on the large number of sensitive PCR assays performed. A general method of hybridization detection has been developed in maize to alleviate the difficulty of finding an insertion allele for a gene of interest. This methodology can be used for any insertion population. The technique involves the simultaneous amplification of insertion flanking sequences (Mu transposon in maize) in a pool of plants and subsequent hybridization of a sequence of interest to the arrayed amplified products. Detection of the amplified product identifies the F1 plant carrying the insertion allele. The 2 dimensional pooling strategy (presented here) allows for the immediate identification of the F2 seed carrying the insertion. Subsequent verification in the F1 pools and in the F2 plants is carried out to distinguish germinal insertion alleles. The method has proven to be an efficient and rapid method of reverse genetics in maize.

W10 Genome Mutagenesis Utilizing Ac in Maize

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The Activator (Ac) family of transposable elements offers several advantages as an insertional mutagen in maize. Nevertheless, the relatively low forward mutation rate associated with Ac has limited its use in large scale mutagenesis experiments. In this project we will exploit the tendency of Ac to move to closely linked sites in the genome to provide a tool for regional mutagenesis. By distributing Ac elements at 10 cM intervals throughout the maize genome, any mapped gene, EST or QTL will serve as a target for Ac mutagenesis. (https://www.fastlane.nsf.gov/servlet/showaward?award=0076892). Each Ac will be maintained in an inbred W22 germplasm as a single active element at a well-defined genetic and physical map position. By exploiting the well-characterized genetics of Ac, we have been able to complete the first step in this project, distributing approximately 100 transposed Ac elements (tr-Ac,s) throughout the maize genome. Work is now underway to precisely define Ac insertion sites through a combination of molecular and genetic mapping techniques. The second step in this project is to utilize the mapped elements in yet another round of transposition selection to generate a collection of approximately 200 lines. Each line will contain a single active element at a well-defined map position for use in targeted gene disruption experiments. To generate transpositions, a simple genetic cross is performed to rapidly identify new transposition events. Insertions into genes of interest can be identified through either forward- or reverse- genetic screens. All seed stocks will be maintained and distributed (without restrictions) at the Maize Genetics Cooperative Stock Center at the University of Illinois.

W11 Agrobacterium-mediated transformation of maize Hi II immature zygotic embryos using a simple binary vector system

Frame, Bronwyn R.(1); Zhang, Zhanyuan(1); Xiang, Chengbin(1); Chikwamba, Rachel K.(1); Shou, Huixia(1); Fonger, Tina (1); Pegg, Sue-Ellen(1); Wang, Kan(1) (1) Iowa State University, Ames, IA 50011-1010, USA

Agrobacterium-mediated transformation of maize Hi II immature zygotic embryos using a simple binary vector system B Frame, Z Zhang*, CB Xiang1, R Chikwamba, H Shou, T Fonger, SE Pegg#, K Wang Plant Transformation Facility, Department of Agronomy, and 1Department of Botany, Iowa State University, Ames, IA 50011 *Current address: University of Missouri, Columbia, MO 65211 # Current address: Western Illinois University, Macomb, IL 61455 Production of transgenic maize using the super binary vector system (Ishida et al., 1996) and the protocol described by Zhao et al. (1999) is currently achieved at a routine efficiency of 4.3% at the ISU Plant Transformation Facility. Because we are not licensed to use the super binary system for other than the gus-marker gene, our aim is to develop a non-super binary (or simple binary) Agrobacterium protocol to produce transgenic maize. To date, we have obtained putatively transformed events from three experiments in which maize Hi II immature zygotic embryos were infected with an Agrobacterium simple binary vector (Agrobacterium strain EHA101 harboring a simple binary vector with the bar and gus genes each driven by a 2x 35S promoter). Southern blot hybridization and histochemical gus assays indicate that the transformants are positive for the transgenes. Further molecular and progeny analysis are currently underway. The protocol and transformation parameters will be discussed. Ishida, Y., Saito, H., Ohta, S., Hiei, Y., Komari, T., Kumashiro, T. (1996). High efficiency transformation of maize (Zea mays L.) mediated by Agrobacterium tumefaciens. Nature Biotech. 14: 745-750. Zhao, Z-Y, Gu, W, Cai, T, Piece, D. (1999). Methods for Agrobacterium-mediated transformation. US Patent # 5,981,840.

W12 Identification and uses of single nucleotide polymorphisms (SNPs) in maize
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Maize Single Nucleotide Polymorphism project at DuPont and Pioneer is aimed at evaluating the potential of SNPs as the next generation genetic marker capable of very high throughput genotyping for genetic mapping, marker assisted breeding and plant germplasm protection. We are using both experimental and bioinformatic approaches to SNP identification. First, we analyzed sequence conservation in 3'-untranslated segments of maize genes. Random cDNA clones from DuPont EST collection were selected for analysis. Primers were designed to amplify about 300 bp preceding the polyadenylation site. Genomic DNA from a collection of over 30 maize lines, representative of the North American corn germplasm, was used as amplification templates. Amplification products were directly sequenced using dye terminator chemistry. The alignment and analysis of amplification products from 20 loci randomly distributed in the genome reveal very frequent polymorphisms, including 1 single nucleotide change per 70 bp, 60% of the SNPs are transitions, and 40% are transversions. One insertion/deletion was detected per 160 bp. These calculations include all changes detected. Comparison of any two lines, for example B73 and Mo17 reveals lesser, but still high degree of polymorphism. Selected ESTs have been genetically mapped using Pyrosequencing. Interestingly, examination of the patterns of nucleotide changes between different corn lines reveals conservation of haplotypes spanning up to 300 bp or more. Only a limited number of conserved haplotypic arrangements was found, demonstrating at the sequence level the severe domestication bottleneck. The conserved haplotypes are expected to reflect the genetic constitution of the ancestral individuals. These experiments allowed us to choose a set of 8 maize lines that represent maximum allelic diversity within the genotypes evaluated. This set of 8 genotypes was used to catalog SNP alleles at 500 loci selected from ESTs and genes of applied interest. Separately, we are extracting SNP information from public and private EST sequences. To this end, EST sequences are assembled into contigs, and SNPs are identified using PolyBayes software (G.Marth, Washington University). Such candidate eSNP have to be confirmed experimentally. All SNPs information is collected in a maize SNPBase, a data base developed for this purpose.

W13 A functional genomics approach to endosperm development
McCarty, Donald R(1); Settles, A. Mark(1); Hannah, L. Curt(1); Koch, Karen E.(1); Messing, Joachim(2); Larkins, Brian(3); Becraft, Phil(4) (1) University of Florida; (2) Rutgers University; (3) University of Arizona; (4) Iowa State University

We describe a multi-institution collaborative program for comprehensive functional genomics analysis of the endosperm. Our strategy involves four essential steps: 1) Construction and screening of a large inbred transposon tagging population, UniformMu, for endosperm mutations. Uniform Mu is specifically tailored for near-saturation transposon mutagenesis of endosperm development. 2) Sequences flanking the Mu insertions contained in each mutant line are extracted using an efficient Mu-TAIL PCR technique. 3) An augmented endosperm cDNA microarray will be constructed from existing maize EST's and a complementary set of new ESTs derived from subtracted endosperm libraries. 4) The PCR amplicons from each tagged endosperm mutation are hybridized to the microarray. Mutant lines that hybridize to the same clone define a "hybridization group". Hence, each hybridization group identifies a set of presumptive independent alleles of a gene involved in endosperm development. Mutations within each hybridization group are resolved into actual complementation groups by genetic tests. As the number of mutations analyzed approaches statistical saturation, all genes represented on the array that have discernible endosperm phenotypes are identified. Given a suitable physical representation of the maize genome (i.e. a cDNA or BAC array), this general strategy may be applied any type of phenotypic variation that can be tagged with a transposon.

Comparative genomics of Zea and Arabidopsis from a colleciton of full length cDNAs
Ken Feldman, CERES
No abstract.

W15 Maize Genomics and MaizeDB
Coe, E(1) (1) University of Missouri-Columbia; USDA Agricultural Research Service, University of Missouri-Columbia (3) Iowa State University

Refer to poster abstracts: **P64 and P69**

W16 Long microsatellites in corn genome
Ananiev, Evgueni(1); Lorentzen, Jen(1) (1) Pioneer Hi-Bred International, Inc. Johnston IA

Distribution and structure of different types of microsatellites were analyzed in corn genomic BAC library. Long stretches of microsatellites were identified which have different levels of complexity. Some long microsatellites revealed extensive polymorphism among different corn inbreds as well as a noticeable level of instability among individual plants of the same inbred line. This is the first observation of dynamic mutations in plants associated with SSR loci. We suggest that long simple sequence repeats can be predisposed to dynamic mutations in plants, as they are in mammalian genomes, and can be sensitive to both genotype- and environmental-specific factors.

W17 Maize Gene Discovery Project: Microarray Production and Analysis
Galbraith, David W.(1); Chandler, Vicki L.(1); Elumalai, Rangasamy(1); Pierson, Elizabeth(1); Decianne, Dominic(1); Walbot, Virginia(2); Fernandes, John(2); Brendel, Volker(3); Gai, Xiaowu(3) (1) University of Arizona; (2) Stanford University; (3) Iowa State University

Maize Gene Discovery is an NSF funded Plant Genome Research Project. The goals of the project are (i) to discover maize genes using EST sequencing and engineered Mu (RescueMu) tagging, and (ii) to develop new tools to facilitate gene mapping and phenotypic analysis. ZmDB, based at Iowa State University (http://www.zmdb.iastate.edu), is the repository and analysis tool for sequence, expression, and phenotype data generated in this project. The site also provides online means for ordering the materials generated in this project, including EST clones, seeds of mutant plants, and microarrays of amplified EST and genomic DNA sequences. As part of this project, over 70,000 ESTs from 14 different libraries have been sequenced and deposited into GenBank by Stanford University. After transfer to the University of Arizona, the ESTs are PCR amplified, and the amplicons characterized by gel electrophoresis. Microarrays are then printed. Data concerning the microarrays are posted to ZmDB, including the quality and length of PCR products, the content, production methods, and format of microarrays, user information, and laboratory and array analysis protocols. Microarrays comprising ESTs derived from endosperm and ear tissue libraries are available for the academic end-user. Microarrays comprising ESTs derived from root, mixed adult, and leaf primordial tissues are currently in production. A unigene set of ESTs is being assembled and resequenced for subsequent microarraying. We present information on the contents and reproducibility of hybridization of the available microarrays.

P1 Differences in 3-PGA Activation Between Potato Tuber and Maize Endosperm ADP-glucose Pyrophosphorylase

Burger, Brian T(1); Cross, Joanna MF(1); Hannah, L. Curtis(1) (1) University of Florida, Gainesville, FL 32611, USA

The allosteric properties of ADP-glucose pyrophosphorylase (AGP) have been well documented in a wide variety of organisms. In plants, AGP is allosterically activated by 3-phosphoglycerate (3-PGA) and inhibited by inorganic phosphate (Pi). This allosteric regulation is physiologically relevant. A mutant of E.coli AGP with altered allosteric properties, glgC-16, increased starch levels by 30% when expressed in potato tubers (Stark et al., Science 258:287). Additionally, Giroux et al. (Proc. Natl. Acad. Sci.USA 93:5824) demonstrated that a Ds-induced insertion in an allosterically important region of the maize endosperm-specific gene shrunken2, encoding the large subunit of AGP, conditioned an 11-18% increase in seed weight in a single dose. However, despite the similarities regarding the allosteric effector substrates, the degree of activation varies widely between plant species. In a clear dichotomy, cereal endosperm AGPs are relatively insensitive to 3-PGA compared to all other plant AGPs. Whether this insensitivity results from an inability to be activated by 3-PGA or reflects the fact that the endosperm AGP is less dependent on 3-PGA for activity has remained an important unanswered question. Here we used size-selected, enzymatically active, E. coli-expressed maize endosperm and potato tuber AGP to address this question. Active fractions were assayed in the presence and absence of allosteric effectors. The relative amount of active protein was measured by use of antibody raised against a 20 amino acid peptide present in both the potato and maize AGP small subunits. In the presence of 3-PGA, rates of catalysis per active protein of the two AGPs were comparable whereas the maize AGP exhibited at least 10-fold greater activity in the absence of the activator. Hence, the maize endosperm AGP exhibits less dependence upon 3-PGA for activity.

P2 Temperature Sensitive Mutants of Maize Endosperm ADP-glucose Pyrophosphorylase

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Increased temperatures reduce yield in the major cereal grains, including maize. This yield loss appears to be due to a decrease in seed weight. For example, Singletary et al. (Aust. J. Plant Physiol. 25:173) showed, using an in vitro kernel development system, that seed weight decreases as temperatures rise from 22ºC to 36ºC. Furthermore, in monitoring starch biosynthetic enzymes and their transcripts in developing, heat-stressed kernels, AGP was the most adversely affected by elevated temperatures (Duke and Doehlert, Environ. Exp. Botany 36:199). Thus, more stable variants of AGP may have significant agronomic ramifications. Previously, this lab identified a His-to-Tyr mutation in the large subunit of maize endosperm AGP that conditions retention of 76% activity after heat treatment at 60ºC for 5 minutes (Greene and Hannah, Proc. Natl. Acad. Sci.USA 95, 13342-13347, 1998). Bacterial expression of AGP combined with a novel mutagenesis scheme allowed us to identify temperature sensitive mutants of the large (shrunken2) subunit of maize endosperm AGP. Two such mutants, sh2ts48 and sh2ts60, fully complement the E. coli glgC- (AGP) mutation at 37°C, but not at 30°C. We mutagenized these mutants and isolated second site suppressor mutations that restored glycogen synthesis at 30°C. These mutants, separated from their respective parental mutation, have been analyzed singly and in combination with the original His-to-Tyr mutation. These analyses will be presented.

P3 Characterization of the maize geranylgeranyl pyrophosphate synthase (GGPPS) gene family
Cervantes-Cervantes, Miguel(1,2); Wurtzel, Eleanore T.(1,2) (1) Lehman College-CUNY, 250 Bedford Park Boulevard West, Bronx, NY 10468, USA; (2) Graduate School and University Center-CUNY

Isoprenoid synthases are a family of enzymes whose main difference is the length of their substrate and products. GGPPS catalyzes the synthesis of the C_{20} isoprenoid geranylgeranyl pyrophosphate, the precursor of many secondary metabolites, including terpenoids, phytoalexins, carotenoids, and prenyl groups. In *Arabidopsis*, various GGPPS isoforms have been found associated with different subcellular compartments, namely, the mitochondria, the chloroplast, the endoplasmic reticulum, and the cytoplasm. Using a heterologous functional complementation assay in *Escherichia coli*, we isolated several cDNA clones coding for GGPPS from a maize B73 endosperm cDNA library with a prevalence of 6×10^{-4}. The 5' end was missing from the clones, hence it was not possible to distinguish if they came from individual messages transcribed from a gene isoform, or from differential processing of a single message. A northern blot using RNA from a maize B73 endosperm developmental series, with one of the longest clones as a probe, showed a single, 1.5-kb transcript appearing between 10 and 15 DAP. Ten clones from a maize B73 BAC library, with a genomic equivalent of 5.6X, were positive when hybridized to one of the larger *Gpps* cDNAs. BAC clones were digested and analyzed by Southern blotting, using either a *Gpps* cDNA 5' 422-bp fragment or a 5' 33-mer as probes. Based on their hybridization patterns, all clones could be classified into one of at least three groups, coinciding with mapping data.

P4 Zymogram Analysis of Starch Metabolic Enzyme Activities in Developing Wild-type and Mutant Kernels
Colleoni, Christophe(1); Marsh, Rebekah(1); Myers, Alan(1); James, Martha(1) (1) Iowa State University

Native PAGE activity gel (i.e., zymogram) analysis is a useful technique for detecting the activities of specific enzyme isoforms, and for making relative comparisons. In particular, it is appropriate for visualizing the activities of starch-metabolizing enzymes, which modify the interaction of a starch-iodine complex. In this study, zymogram analysis was used to profile the activities of starch metabolic enzymes from wild type maize kernels in two inbred backgrounds, W64A and Oh43, as well as various mutants that had been introgressed into these backgrounds. Total proteins from kernels harvested at mid-development and separated on native PAGE gels were transferred to starch containing gels and stained with iodine. Two-dimensional zymogram analysis, in which proteins were first fractionated by FPLC, provided improved resolution for activity bands. In some instances, immunoblot analysis was used to correlate specific enzyme activities with specific polypeptides. Slight differences were seen in the enzyme profiles of the two inbred lines, as evidenced by the migration of BEI activities as either three or four distinct bands. In both lines, BEIIa and BEIIb activities were seen as single bands. Comparisons of the enzyme profiles among ae and su3 mutants, and allelic series of su1 and du1 mutants, revealed that these mutations condition allele-specific pleiotropic effects on other starch metabolic enzymes. These results indicate that quarternary structure and conformation may impact enzyme functionality, and suggest that particular starch biosynthetic enzymes form functional complexes.

P5 Allelic Effects of Maize Starch Debranching Enzyme Genes *sugary1* and *zpu1*

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The structural organization of granular starch in maize kernels is determined by the coordinated activities of starch synthases, branching enzymes, and debranching enzymes (DBEs), although the specific roles of DBEs are not yet clear. Two distinct DBEs have been identified in maize endosperm tissue, both of which have been highly conserved throughout the evolution of higher plants. The *sugary1* (*su1*) gene codes for an isoamylase-type DBE that affects the other, a pullulanase-type DBE that is the product of the *zpu1* gene. Because both DBEs are deficient in *su1* mutant kernels, it is not clear whether one or the other or both are responsible for the *su1* phentotype. Several allelic mutations of *su1* condition kernel phenotypes of varying severity. In this study, we investigated the molecular basis for six of these *su1* alleles. The observation of numerous allele-specific effects on starch structure and the activities of other starch modifying enzymes implicates both enzymatic and non-enzymatic features of the SU1 polypeptide in starch biosynthesis. Also, using the Pioneer TUSC system we identified a *Mu*-induced mutation in the *zpu1* gene. Analysis of this mutation, *zpu1-204,* revealed it is a null allele, in which *zpu1* transcript and ZPU1 activity are missing from the developing endosperm. Kernels homozygous for *zpu1-204* are phenotypically similar to wild type kernels, suggesting that the pullulanase-type DBE is not required for normal starch accumulation. This indicates that *su1* kernel phenotypes are due to specific alterations in the SU1 isoamylase-type DBE, and that loss of pullulanase-type DBE activity most likely occurs as a secondary effect of the *su1* mutation. We predict that the pleiotropic effects of the *su1*- mutations are indicative of fundamental aspects of the starch biosynthetic mechanism.

P6 A novel homolog of Bt2, the small subunit of ADP Glucose Pyrophosphorylase, inhibits carotenoid accumulation.

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Carotenoids, derived from plant food sources, are converted in humans to vitamin A and other important compounds needed for growth and development. Endosperms of food crops, such as maize and wheat, are low in carotenoid content, and are especially low in provitamin A as compared with nonprovitamin A carotenoids. In an effort to metabolically engineer this pathway in maize endosperm, we are screening for genes that either promote or interfere with carotenoid accumulation. We are using a heterologous system in which carotenoid genes from the bacterium Erwinia uredovora are introduced into Escherichia coli cells that are subsequently transformed with a maize cDNA expression library; these doubly transformed cells are then screened for alterations in carotenoid accumulation. We have isolated a novel cDNA that negatively impacts carotenoid accumulation in E. coli. Based on DNA sequencing and further functional testing, we have determined that this cDNA encodes a novel form of the small subunit of ADP Glucose Pyrophosphorylase (ADPG), a rate-limiting enzyme in starch biosynthesis that has also been of interest in efforts to enhance starch content in plants. By complementation analysis, we demonstrate that this cDNA encodes an ADPG small subunit Bt2 homolog that has a unique effect on carotenoid accumulation, not found for either Bt2 or Sh2, the large maize ADPG subunit. Based on our results, we will discuss the possible impact of simultaneously manipulating the starch and carotenoid biosynthetic pathways.

P7 Proteomic analysis of embryonic and postembryonic root formation in maize
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The mutant lrt1 is specifically affected in early postembryonic root development in that it fails to initiate lateral roots on the embryonic primary and seminal roots (Hochholdinger and Feix (1998): Plant J. 247-255). In an effort to further characterize embryonic root formation and the initiation of postembryonic lateral roots, protein patterns of wild-type and lrt1 mutant seedling-roots were analyzed and compared via proteomics technology. Proteomics combines the resolution of large two-dimensional protein gels with the recent improvements in mass spectrometry to identify large numbers of proteins isolated from specific organs, tissues, cells or even organelles. Total root proteins were isolated from the primary and seminal roots of 10-day-old wild-type and lrt1 mutant seedlings that had already formed postembryonic lateral roots. Proteins were separated in two dimensions according to their isoelectric point and mass and stained with coomassie blue. Several differences in protein accumulation were observed between the wild-type and lrt1 mutant roots. Each of the separated proteins was isolated and digested with trypsin. The resulting peptide fragments were analyzed using a MALDI TOF mass spectrometer and compared to the predicted trypsin fragments from proteins in public databases. Using this approach it was possible to separate about 150 proteins and identify 116 of these.

P8 Characterization of eEF1A protein isoforms in developing maize endosperm
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Elongation factor 1A (eEF1A) is a multifunctional protein in eukaryotic cells. In maize (Zea mays L.) endosperm, eEF1A co-localizes with actin around protein bodies, and its concentration is highly correlated with the protein-bound lysine content, even though the protein itself accounts for only 1-3% of the total lysine. eEF1A is encoded by multiple genes in maize; five of these genes are expressed in the endosperm tissue, and two account for about 75% of the mRNA transcripts. In eukaryotes, eEF1A is subject to several types of post-translational modifications that affect its activity and subcellular location. To understand the biological basis of the correlation between eEF1A and endosperm lysine content, we are investigating the roles of different forms of eEF1A protein. Three different eEF1A isoforms were purified from endosperm tissue of the maize W64A inbred by high performance liquid chromatography (HPLC). Interestingly, there is a gradual change in the relative abundance of these isoforms during endosperm development (11-20 DAP), suggesting that their accumulation is developmentally regulated. Furthermore, the eEF1A isoforms differ in their ability to associate with actin, suggesting that they are functionally distinct. Peptide maps obtained from the three different isoforms are very similar. However, for each isoform at least two eEF1A proteins have been identified, suggesting that their differences in functionality are due to post-translational modifications. Experiments are in progress to characterize further the eEF1A isoforms with regard to post-translational modifications and biological activities to understand their significance in maize endosperm development.

P9A Cloning and characterization of a phytoene synthase (Psy) gene from rice and comparison to maize Y1

Matthews, Paul D. (1); Wurtzel, Eleanore T.(1) (1) Lehman College and The Graduate School of the City University of New York, Bronx, NY, 10468, USA

Carotenoids serve as light-harvesters, photoprotectors, oxidative-stress protectors, colorants, and metabolic precursors to hormones and odorants. Phytoene synthase (PSY) is the branch-point enzyme in the poly-isoprenoid biosynthetic pathway that dedicates geranygeranyl pyrophosphate to carotenoids and carotenoid catabolites such as apocarotenoids and ABA. Thus, PSY is likely to be a rate-controlling enzyme in carotenogenesis in leaves, fruits, flowers and endosperms. PSY transcript levels, protein accumulation, or enzymatic activity have been shown to be increased concomitant with carotenoid accumulation in a variety of tissues among model plants, such as daffodils, bell peppers, tomatoes, and maize. Using ESTs and BAC clones we have cloned and characterized a Psy gene from rice. The rice gene shares identical exon structure with maize Y1(Psy), which was cloned and characterized by Brent Buckner (Buckner, B and Robertson, DS. 1990. Maize Genetics Conference Abstracts 32). Rice Psy also shares exon structure with tomato Psy1. Hydrophilicity plots and chloroplast transit peptide cleavage prediction for the rice PSY deduced protein primary structure, suggest a cleavage site at amino acid residue 58. This prediction yields a mature protein with a molecular mass of about 38 kDa, which confirms previous findings based on Western blotting for maize (Yu, J and Wurtzel, E. 1999. Maize Genetics Conference Abstracts 41) and rice amyloplast proteins. Phylogenetic analyses, analyses of ESTs, chromosome mapping and rice-maize syntenic relationships will be used to suggest that there may be two Psy genes in rice and that the gene sequence reported here is not the ortholog of maize Y1.

P9B Maize phytoene desaturase (PDS) and zeta-carotene desaturase (ZDS) produce poly-Z-lycopene: Implications for genetic manipulation of carotenogenesis in maize and rice

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TALK #14 ABSTRACT

THE SEED

SATURDAY MORNING

P10 Invertase inhibitor homologs (inhh) in maize

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In higher plants, acid invertases in the cell wall and in the vacuole perform important metabolic functions but are also involved in plant development and in defense reactions (1). While transcriptional up-regulation of invertase expression in response to different cues is well characterized, the equally required silencing of invertase function is less well understood. In addition to transcriptional down-regulation, another mechanism may operate via invertase inhibitor proteins (INH). Although biochemical studies on INH proteins in dicot and monocot plants were already reported more than 30 years ago (2,3), molecular analysis, including cloning and proof of function in vitro and in planta are thus far restricted to tobacco and potato (4,5). INH proteins are believed to play a critical role in posttranslational modification of invertase activity through protein-protein interactions. Like for acid invertases, evidence was obtained for cell wall and vacuolar isoforms. From the rapidly increasing maize EST database, we could identify three clones encoding proteins with significant sequence homology to dicot Inh genes. For one of these clones (Zm-inhh1; inh homolog), we have generated recombinant protein. Surprisingly, recombinant Zm-INHH1 protein stimulated invertase activity in vitro (up to 4.5-fold, depending on the invertase preparation). Zm-inhh1 appears to be preferentially expressed in anthers and pollen. With an antiserum directed against Zm-INHH1 protein, we could identify in anther and pollen extracts a protein of expected size, the identity of which was confirmed by MALDI-TOF analysis. Further studies are under way to explore the role(s) of the different maize Inh homologs during plant development. References: 1. Sturm & Tang (1999) TIPS4: 401-407; 2. Pressey (1967) Plant Physiology 42: 1780-1786; 3. Jaynes & Nelson (1971) Plant Physiology 47: 629-634; 4. Greiner et al., (1998) Plant Physiology 116: 733-742; 5. Greiner et al., (1999) Nature Biotechnology: 708-711.

P11 Maize endosperm secretes a novel antifungal protein into adjacent maternal tissue

Serna, Antonio (1); O´Connell, Timothy(1); Maitz, Monika(1); Santandrea, Geraldina(1); Thevissen, Karin(2); Tienens, Koenraad (2); Hueros, Gregorio(1); Faleri, Claudia(3); Cai, Giampiero(3); Lottspeich, Friedrich(4); Thompson, Richard(1) (1) (1)MPI für Züchtungsforschung, Köln, D; (2) (2)Kathioleke Universiteit Leuven, B; (3) (3)Dept. Environmental Sciences, Univ. Siena, I.; (4) (4)MPI für Biochemie, Martinsried, D.

A series of endosperm transfer layer-specific transcripts has been identified in maize by differential screening of a 10 days after pollination (DAP) cDNA library. Sequence comparisons revealed among this class of cDNAs a novel small gene family of highly diverged sequences encoding Basal layer Antifungal Proteins (BAPs). The bap genes mapped to two loci on chromosomes 4 and 10. bap-homologous sequences have thus far only been detected in maize, teosinte and sorghum, and are not present in grasses outside of the Andropogoneae tribe. BAP2 is synthesized as a preproprotein, and is processed by successive removal of a signal peptide and a 29-residue prodomain. The proprotein can be detected exclusively in microsomal membrane-containing fractions of kernel extracts. Immunolocalization reveals BAP2 to be predominantly located in the placentochalazal cells of the pedicel, adjacent to the Basal Endosperm Transfer Layer, (BETL) cells, although the BAP2 transcript is only found in the BETL cells. The biological roles of BAP2 propeptide and mature peptide have been investigated by heterologous expression of the proprotein in E. coli and tests of its fungistatic activity, and that of the fully processed form, in vitro. The mature BAP2 peptide exhibits potent broad-range activity against a range of filamentous fungi, including several plant pathogens.

P12 A closer look at an allegedly recessive mutation: dose effects at the brown midrib1 locus
Vermerris, Wilfred(1); Thompson, Karen J.(1); McIntyre, Lauren M.(1) (1) Purdue University, West Lafayette, IN 47907, USA

The brown midrib1 mutant of maize was discovered in 1924 and was the first of the four currently known brown midrib (bm) mutants. These mutants, with their characteristic brown vascular tissue in the leaves and stem have been shown to affect cell wall composition, and the mutant alleles have always been considered recessive. Based on initial field observations we decided to analyze the flowering dynamics of the brown midrib mutants in depth. Our studies indicated that the bm2 and bm4 mutants flowered significantly later than the wild-type control, whereas the bm1 mutant flowered earlier. Surprisingly, the bm1 heterozygote flowered even earlier, despite the lack of a brown midrib phenotype. In a follow-up chemical study the cell wall composition of vascular tissue was analyzed with Fourier transform infrared spectroscopy. This indicated that the bm1 heterozygote could be distinguished chemically from the wild type and bm1 homozygote. The combination of the chemical and flowering data provides evidence for dose effects at the bm1 locus. This finding adds an additional mechanism to introduce variation in the composition of the cell wall, and may provide an incentive to take a closer look at other allegedly recessive mutations.

P13 Analysis of the Fatty Aldehyde Dehydrogenase Genes of Maize via Denaturing HPLC-based Reverse Genetics
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The aldehyde dehydrogenase (ALDHs) gene family has more than one hundred and forty members and includes mitochondrial, cytosolic and microsomal isozymes. In plant, the *rf2a* gene encodes a mitochondrial ALDH that is involved in the restoration of fertility to cms-T maize. Unlike many other well-characterized family members, the physiological functions of the microsomal fatty ALDHs are not well understood, even in the relatively well-studied human system. Although no plant mutants lacking fatty ALDH activity have been described, it is known that in humans a deficiency of fatty ALDH activity causes the genetic disorder Sjogren-Larsson syndrome characterized by ichthyosis, mental retardation, spasticity, and accumulation of 16- and 18-carbon saturated alcohols in the plasma. The maize genome contains four putative fatty ALDH genes. The possible biological roles of fatty ALDHs include the detoxification of the reactive aldehydes produced during lipid peroxidation and fatty alcohol metabolism. Denaturing HPLC (dHPLC) is being used to identify EMS-induced mutants in these genes.

P14 Mitochondrial function is required for the accumulation of normal level of photosystem I during chloroplast biogenesis in maize

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Organelle interactions are integrated with cellular signaling networks and are involved in many aspects of plant biology. We are trying to understand the molecular mechanisms underlying the interplay between mitochondria and chloroplasts. The maize NCS2 (nad4-deletion) and NCS6 (cox2-deletion) mutations provide models to study the requirement of mitochondrial function for chloroplast biogenesis and photosynthesis. We demonstrate that both mitochondrial mutations are associated with a reduction of Photosystem I (PSI) and its activity. Western blotting analysis shows that the loss of PSI correlates with a great reduction of nuclear-encoded PSI subunits (PsaD, PsaE, and LHCIs) and plastid-encoded PsaA, PsaB and PsaC subunits. Northern blotting analysis shows that their mRNA levels are also decreased. The results indicate that the decrease of the PSI proteins is due to suppression of transcriptional and/or post-transcriptional processes. We propose that a signaling pathway senses the dysfunction of the mitochondrial electron transport chain (ETC) and responds by down-regulating PSI assembly in the chloroplast.

P15 The am1-pral and pam1 genes: bouquet formation and homologous synapsis

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We are interested in determining the specific role of meiotic genes in controlling key meiotic events such as initiation of meiosis, search for homology, pairing, synapsis, meiotic recombination, and chromosome segregation. Here, we present the results of our study of two maize meiotic mutants (am1-pral and pam1). These two mutants have been chosen for study because of their dramatic effect on the progress of meiosis: the am1-pral allele arrests meiosis during the leptotene-zygotene transition, and the pam1 gene delays or stops meiosis between leptotene-pachytene. In addition, homologous synapsis does not occur properly (pam1) or at all (am1-pral) in these mutants. These features of the am1-pral and pam1 mutants made them an ideal choice for studying the relationship between the bouquet formation and homologous chromosome pairing and synapsis . The coexistence of normal bouquet formation and complete absence of homologous synapsis in am1-pral mutant meiocytes suggests that the homologous synapsis and bouquet clustering are two independent genetic events. The further investigation of the leptotene-zygotene transition stage in am1-pral meiocytes will allow us to provide "a time-course" experiment for maize meiocytes, and as a result to have a complete picture of the telomere clustering in maize prophase I. The timing of the arrest/delay meiosis by the pam1 gene appears to be at the leptotene/zygotene transient stage rather than at pachytene stage, according to similarity in pattern of bouquet clustering of two mutants and TEM pictures of pam1 meiocytes (unresolved interlocks together with nonhomologous synapsis). Moreover the nonhomologous synapsis in the pam1 mutant could be a result of the long delay at prophase I stage rather than a primary effect of the gene on the search for homology.

P16 FISH POSITIVE PROBES IN MAIZE

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In maize meiotic cells, chromosome identification is possible using the morphology of the chromosomes. In maize somatic cells, however, chromosome identification is very difficult because of the condensed state of chromatin. Current progress in fluorescent in situ hybridization (FISH) technology provides the potential to identify all maize somatic chromosomes. Several repeated sequences have been available (knob, Cent C, Cent 4 and 5S etc.) to detect FISH signals on maize chromosomes. By using them as FISH probes and combining size, arm ratio, and the presence of the satellite, identifying all the ten chromosomes is still time consuming and difficult in maize somatic cells because of the polymorphic nature of knobs (which alters size and arm ratio). To develop a system to identify all maize somatic chromosomes, we have been conducting a screen of DNA clones, which show chromosome specific FISH signals. Currently we have identified twenty FISH positive clones. Among them, eight clones show four signals on three chromosomes at identical positions, five clones show one signal at the knob region of chromosome 6 , six clones show signals at centromeric regions, and one clone shows signals at telomeric regions on several chromosomes.

P17 Physical mapping to maize chromosome by using a complete set of oat-maize addition lines

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All ten different maize chromosomes were recovered as single additions to haploid oat among 139 maize-positive F1 plants generated from about 60,000 oat x maize crosses. Doubled haploid addition plants (2n = 6x+2 = 44) were produced among the F2 offspring from F1 plants either naturally by meiotic restitution or artificially by colchicine application for maize chromosomes 1, 2, 3, 4, 5, 6, 7, 8, and 9. To date a total of 33 fertile addition lines are maintained to produce seed with seven lines each for chromosome 2 and 4, six lines for chromosome 9, four lines for chromosome 7, two lines each for chromosome 3, 5, 6, and 8, and one line for chromosome 1. Maize chromosome 10 has been recovered as an addition to a haploid oat and is vegetatively maintained in the form of tiller-clones; because F2 offspring have not yet been produced, seed is not available. However, genomic DNA from the chromosome 10 addition plant is available to the scientific community. Here we report on the specific characters of these additions and demonstrate their usefulness for mapping genes and markers to chromosome, in particular for high throughput applications like microarray technology. Microarray slides immobilize unlabeled DNA probes to glass, which allows hybridization with labeled DNA. We are optimizing this technology to use labeled genomic DNA from our addition lines to allocate sequences to chromosome(s). This material is based upon work supported by the National Science Foundation under Grant No. 9872650.

P18 Discovery and Characterization of Maize Alternatively Spliced Genes by EST Analysis
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see TALK #23 ABSTRACT

THE GENE

SUNDAY MORNING

P19 Tree Whittling
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Phylogenomics is a method of sequence-based function prediction by phylogenetic analysis (Eisen 1998). The phylogenomic method often yields more accurate functional hypotheses than techniques based solely upon sequence similarity (such as BLAST). It is implemented by constructing a reasonable phylogenetic tree for a given dataset, then mapping the functions of experimentally analyzed proteins onto the tree. Kuhner and Felsenstein (1994) showed that the optimality criterion most successful at inferring accurate phylogenies overall is maximum likelihood (ML). However, the ML heuristic search algorithms currently available are computationally impractical for large datasets (especially those consisting of protein sequences). We are developing a ML heuristic search tool that we call Whittle. Whittle progressively jackknifes an alignment to generate multiple neighbor joining trees, then compares those trees statistically on the basis of their relative likelihood scores. This sampling procedure finds phylogenetic trees that are similar to those built by ML star decomposition, but requires only a fraction of the computations.

P20 Using FRET to refine the order of components in the maize kinetochore/centromere in meiosis and mitosis

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Fluorescence Resonance Energy Transfer (FRET) is a combination of microscopic and mathematical techniques that can determine the proximity between molecules separated by roughly 10-100 A, a scale not possible using ordinary light microscopy alone. We studied the kinetochore/centromere complex using maize root tips sections and meiocytes, a combination of immunolocalization and in situ hybridization, and fluorescence light microscopy. Image sets from the microscope were analyzed for grey value content and a spreadsheet model based on ratios between values provided a quantifiable value of FRET. We have shown that CENP-C and a maize satellite DNA repeat CentC are separated by a 200-300 A limit as there is a positive FRET value associated. Combinations of antibodies to CENP-C, MAD2 or tubulin alone also showed positive FRET, while various combinations of tubulin, CENP-C, MAD2, and satellite DNA showed reduced FRET (components separated by more than 300 A). The same general trends were observed for both meiosis I and mitosis, with statistical measures used for further analysis sho that the controls and CENP-C/satellite DNA grouped together, while the other combinations fell into other groups. From the different paired results it is possible to order the components according to the relative proximity shown by FRET.

P21 dsy498: a new meiotic gene affecting bouquet formation and homologous chromosome synapsis

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Our goal is to determine the specific functions of maize genes controlling key events in meiosis. desynaptic498 (dsy498) is a new mutation affecting chromosome synapsis, not allelic to other known desynaptic mutations in maize. Mutant plants homozygous for dsy498 exhibit a complete failure of homologous chromosome synapsis; 20 univalents are present in most meiocytes at diakinesis and metaphase I. Transmission electron microscopy studies of spreads of whole-mount prophase I nuclei confirm the absence of homologous synapsis. Twenty unsynapsed axial elements are seen in most zygotene ˆ pachytene nuclei. The very rare synaptic structures are mostly the result of nonhomologous synapsis of chromosomes folding back on themselves. 3-D microscopy using fluorescent in situ hybridization (FISH) with the telomere probe demonstrates abnormal bouquet formation at the zygotene stage in mutant plants. In contrast to telomeres forming a bouquet by clustering on a single region of the nuclear envelope in normal maize plants, in dsy498 mutant meiocytes either multiple telomere clusters or single clusters accompanied by several telomeres located elsewhere in the nucleus are found. The presence and distribution of Rad51 recombination protein previously has been correlated with homologous chromosome synapsis in normal maize plants. Preliminary immuno-fluorescence results indicate the presence of very few and abnormal RAD51 foci in the mutant meiocytes at the zygotene stage, in contrast to several hundred foci present in wild-type plants. The dsy498 gene, therefore, may be involved in recombination in addition to affecting homologous chromosome synapsis and bouquet formation. The dsy498 mutation was isolated from an active Mutator stock and we are currently in the process of cloning the gene.

P22 Towards an integrated cytogenetic map of maize
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Our goal is to generate a cytogenetic map of the maize genome which will integrate information from existing cytological and genetic maps with new cytological and physical data. We are using three-dimensional fluorescent in situ hybridization (3-D FISH), deconvolution light microscopy and computerized image analysis to place genetically mapped genes onto the cytological map of maize pachytene chromosomes. The maize community has generated a wealth of genetically mapped chromosome breakpoints. Much of these data has been collated by Ed Coe, and integrated by David Hoisington. The last maize cytogenetic map was presented in the MNL in 1993. We are updating this classic cytogenetic breakpoint map and overlaying our data obtained by using repetitive and single/low copy sequences as probes to pachytene chromosomes. Here, we present cytogenetic maps for maize chromosomes 1, 2, 4, and 9. These maps are works in progress and will eventually provide information on the distribution of genes and repetitive elements, on the position of recombination events, and possibly shed light on the process of meiotic homologous pairing. They will also be helpful for integrating the many genetic maps of maize, and for the genetic placement of markers that can not be mapped genetically.

P23 Is recombination affected by stress in maize?
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Plant genomes have the capacity to change in response to abiotic stress and other environmental signals. Increased recombination has been proposed as a response mechanism in several species (e.g., Drosophila, Neurospora, Mus and Lycopersicon). In maize, effects on recombination have been documented for sex, genotype, environment, chromosome rearrangements and supernumerary chromosomes. The effects of water stress have not been reported. In this study, we compare male recombination observed in stress and non-stress plants. In the greenhouse, two treatments were applied to F1 plants (B73xMo17), stress (25% field capacity), and non-stress (field capacity). The treatments were applied before and during male meiosis. Backcross populations (93 individuals each) were created by crossing each F1 as male to B73. Two backcross populations (i.e., two F1 plants) from each treatment were analyzed with 16 SSR loci from chromosome one to create genetic maps. Comparisons of recombination were made within and between treatments. No significant differences were observed between maps within a treatment; therefore, maps from the same treatment were combined. However, differences were observed between treatments: the total genetic map length was greater for stressed plants (170.7cM vs. 156.4cM). The long arm of the chromosome one was the region of additional recombination in the stressed plants. A significant excess of Mo17 alleles was observed in the same genetic region of all populations. Analyses of an additional backcross population for chromosome one and the study of another chromosome are being conducted.

P24 Maize ORC genes

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Origin recognition complex (ORC) proteins functioning in gene replication are essential for gene silencing in yeast and animals. ORC consists of 6 subunit proteins that are highly conserved among all species. It is not known, however, whether ORC exists in plants, but individual homologs of ORC1 and of ORC 2 subunits have been reported in rice and Arabidopsis, respectively. Our goal is to characterize the components of a putative complex in a plant (maize). Maize EST clones, corresponding to ORC subunits 1,2,3 and 4 will be studied. The molecular structure of the entire ORC4 gene is currently being established. Potential binding partners of the individual subunits will be isolated through yeast two hybrid system, expressing maize cDNA-library with the ORC-genes as baits. These studies will provide a first look at the structure of ORC in a plant and open possibilities to study a connection between DNA replication, cell growth and gene silencing.

P25 Some new mutants affecting the coleoptile in maize

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Our laboratory has been looking at seedling mutant phenotypes for both the Gene Discovery (V. Walbot, PI) and MTM (R. Martienssen, PI) NSF Plant Genome Projects. Active Mutator males were crossed onto non-Mu lines, and self-pollinated. Our laboratory plants 16 of these F2 seeds on vermiculite, under a thin layer of sand. The coleoptile is readily observed under these conditions and several interesting phenotypes have been observed. We feel these mutants will prove to be important for the study of organogenesis since the coleoptile develops from its own field of cells quite separate from the shoot apical meristem. Further, the nature of coleoptile mutants relates to the general biological concept called "homology." These data are available or will soon be available on the respective project websites: http://www.zmdb.iastate.edu/ and http://mtm.cshl.org/.

P26 SEMAPHORE1 contributes to knox gene regulation in a separate genetic pathway than ROUGH SHEATH2.

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semaphore1-O (sem1-O) is a recessive mutation that induces ectopic expression of the duplicate knox (knotted1-like homeobox) genes rough sheath1 and knox4 (gnarly1) in maize shoots. Homozygous semaphore1 mutants exhibit pleiotropic shoot phenotypes. Mutant seed are reduced endosperm/small embryo kernels that display approximately 50% mortality in inbred B73 genetic backgrounds. Viable sem mutant plants are brachytic (short internode), and sem leaves contain aberrant cell-fate acquisition at the blade/sheath boundary. Directed Mutator transposon-tagging was used to identify two additional mutant alleles, designated sem1-12 and sem1-9. Plants homozygous for these independently-derived sem1 mutations display a more extreme „knox‰ phenotype, and resemble rough sheath2 (rs2) mutants. Genetic analyses reveal an additive phenotype in sem1-O/rs2 double mutant plants, indicating that SEMAPHORE1 and ROUGH SHEATH2 may function in separate genetic pathways to regulate knox gene expression in maize shoots. Previously, a Mu8-tagged genomic fragment that is closely-linked to the sem1-O mutation was isolated and analyzed. However, in approximately 21 kb of genomic DNA surrounding this clone, no transcribed sequences were identified. Therefore, we used a modified AIMS protocol to identify a new MuDR-tagged clone, designated A1-7, that is linked to the sem1-O mutation. The A1-7 clone hybridizes to a transcript in non-mutant maize seedlings that is absent in sem1-O mutants, and identifies a polymorphism linked to the sem1-12 allele. These data indicate that the sem1 gene has been cloned. Analyses of sem cDNA,s are in progress.

P27 A Functional Blueprint for Maize Endosperm Development (ZEASTAR)

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ZEASTAR, an EU funded consortium project involving ten partners (including Syngenta), will examine the transcriptome and proteome of developing maize endosperm. Twenty part-normalised cDNA libraries will be constructed from five key stages of endosperm and kernel development. These key stages will cover: (1) 0-96 hours post fertilisation (free nuclear division and cellularisation), (2) 4-10 days post fertilisation (cell division and differentiation), (3) 12 days to maturity (reserve synthesis), (4) dry down and (5) germination. One thousand clones from each cDNA library will be single-pass sequenced to generate a total of 20,000 EST sequences. Of these, 7,500 unique ESTs will be selected for the construction of high-density microarrays, with which the expression profile of the developing maize endosperm will be characterised. The arrays will be used to compare expression profiles of endosperm grown under varying conditions or from different genotypes. In parallel, 2-D gel electrophoresis will be used to analyse the proteome of developing maize kernels. These two complementary studies will identify ESTs and/or proteins that show highly specific temporal and spatial expression patterns during maize kernel development. Genes of interest will be used to generate kernel or endosperm-specific gene knockouts via Mutator transposon tagging Œgene machine, technology. The biochemistry, protein profile and gene expression patterns of modified and normal endosperms will be compared. Ultimately, mutant maize lines with potentially beneficial yield characteristics may be incorporated into breeding programmes.

P28 tie-dyed1: a non-clonal leaf cell patterning mutant
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The focus of our lab is on understanding the genetic basis of maize leaf development. The leaf is composed of longitudinal cell files that are clonally related. However, within the leaf a cell's position is ultimately more important than its lineage for determining the cell's final differentiation state. These data imply that for a cell to adopt its final differentiated program, cell-cell communication is required to coordinate the process. To understand how this coordination is controlled we have been characterizing a mutation that affects cell patterning in the leaf blade. tie-dyed1 (tdy1) is a recessive mutation that causes non-clonal yellow-green sectors to appear in the leaf blade late in leaf development. Initially mutant leaves are indistinguishable from their wild type siblings. After approximately a week of growth, pale green sectors become visible while the remainder of the leaf blade becomes darker green. The tdy1 sectors are often bounded by lateral veins suggesting the involvement of a diffusable signal. We have used a combination of genetic and physiological experiments to investigate the origin of these non-clonal sectors. To determine what signals tdy1 may be responding to, double mutants were constructed with either longitudinally or transversely striped mutants. These studies suggested that the tdy1 sectoring was independent of both types of signals, as evidenced by the tdy1 sectors crossing over the boundaries of both longitudinal and transverse stripes. Double mutants between tdy1 and maize leaf architecture mutants suggested that incident light is important for expression of the tdy1 sectoring. To further study the role of light, tdy1 plants were grown under low and high light regimes. Under low light no sectors were produced; under high light sectors appeared. Intriguingly, leaves produced under the low light regime did not sector when shifted to high light suggesting a developmental window of competency or a timing component to sectoring. Future investigations will uncover the role of tdy1 in cell-cell communication and in coordinating this developmental decision.

P29 Brick genes are required for epidermal cell lobing in maize.
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Brick genes are required for epidermal cell lobing in maize. To further understand cell morphogenesis, we have isolated mutants in three maize genes which disrupt epidermal cell shape. In brick plants, the cells of the epidermis divide and develop normally, except that during the final phase of cell expansion, marginal lobes fail to form, resulting in smooth, rectangular cells. This defect in cell lobing is specific to the epidermis, as mesophyll cell lobing is unaffected. Mosaic analysis has shown that both brk2 and brk3 are cell autonomous, while brk1 is cell non-autonomous. This suggests that Brk2 and Brk3 act within each cell to control cell shape, while Brk1 encodes or controls the production of a diffusible or transported signal required for cell lobing. Finally, the cytoskeleton has been shown to be involved in lobe formation. In mesophyll cells, microtubule banding guides the deposition of cellulose to defined areas on the cell wall, resulting in periodic wall thickenings. Actin has also been implicated in cell lobing, but currently, its role in this process is unclear. To address the role of the cytoskeleton and cytoskeleton-associated molecules, leaf samples from various developmental stages have been analyzed using a whole mount staining procedure. Before cell lobing occurs in wildtype, no difference is observed in the organization of the cytoskeleton between wildtype and brick; however, at the stage when cell lobing begins to occur in wildtype, the organization of the cytoskeleton and its associated molecules is altered.

P30 Evidence supporting the cloning of branched silkless

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The formation of the complex inflorescence architecture of maize requires the coordinate activities of several meristems, including the branch, spikelet pair, and spikelet meristems. Mutations in several genes can lead to higher order branching within the inflorescence. A mutation in one such gene, branched silkless (bd1), has been described as being unable to switch from spikelet meristem branching to floral meristem production in the ear. As a result, the spikelet meristem continues to branch indeterminately. Thus, the bd1 gene is necessary for the acquisition of spikelet meristem determinacy. We have collected several putatively tagged alleles of branched silkless from both random and targeted tagging experiments. From one of our alleles, designated bd1::mu20250, we have identified a Mu1 element that cosegregates with the branched silkless phenotype in over 100 chromosomes. Through the analysis of our other bd1 alleles, evidence will be provided that the DNA flanking the Mu1 insertion corresponds to bd1. The cloning of bd1 will help us understand how meristem determinacy is controlled within the maize inflorescence.

P31 Evolution of lateral organ identity in land plants

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Normal leaf development in maize (a monocot) is dependent upon the downregulation of knox genes at leaf initiation. Repression of knox then has to be maintained throughout the rest of leaf development. The rs2 gene has been shown to be required for this repression (Tsiantis et al., 1999 and Timmermans et al., 1999). Genes of similar sequence and function to rs2 have been characterised in two dicots, Arabidopsis and Antirrhinum. This conservation of function suggests that the interaction between rs2 and knox arose before the divergence of monocots and dicots. The aim of this work is to identify the phylogenetic level at which rs2/knox interactions were recruited to regulate leaf development. Progress made so far will be presented.

P32 Globby affects development of the apical region of maize endosperm.
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The molecular mechanisms regulating the development of the diploid embryo and triploid endosperm are poorly understood. Mutant analysis provides an effective means of dissecting key developmental programmes, such as those specifying cell fate and differentiation during maize kernel development. In an effort to study this area of development, we have investigated mutant maize kernels with altered morphologies. Several independent lines segregating for a similar recessive defective kernel (dek) mutation were obtained from a regional mutagenesis programme utilising an active Ac element residing on the short arm of chromosome 1. The phenotype varies in severity and consists of small, pitted, globular-shaped kernels, hence the mutation is termed globby (glb). Efforts are in progress to determine linkage of an Ac element to the glb phenotype. Preliminary data shows co-segregation of an Ac element with glb in one of these lines (glb-1). Results presented demonstrate that by 12 days after pollination the mutation is clearly visible. Light microscopy studies reveal that unlike other previously characterised dek mutants, glb-1 appears to affect only apical organisation of endosperm tissue, leaving basal structures intact.

P33 The empty pericarp2 mutants: defective regulation of the heat shock response aborts embryo development at an early stage.
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Empty pericarp2 (emp2) is a recessive, lethal mutant defective in kernel development. Development of the mutant embryo is severely retarded compared to wild type embryos, although it does proceed until the coleoptilar stage whereupon kernel abortion and decomposition ensue. The emp2 mutation maps to chromosome 2L and was cloned by Mu-tagging. The hypothetical protein is identified as HEAT SHOCK BINDING PROTEIN (ZmHSBP1), a negative regulator of the heat shock response, which shows high homology to orthologues from mammals and C. elegans. Genomic sequencing of emp2-O and an independent allele (emp2-PI) obtained using TUSC technology reveals that both contain Mu1 insertions in the first intron of the emp2 locus. Northern gel-blot analyses reveal that a 700 bp transcript is constitutively expressed in all maize tissues tested so far, including: kernels from 4 dap to 18 dap; seedlings; seedling leaves; and vegetative shoot apices. EMP2 transcripts are found in both the recessively-inherited mutant, and non-mutant sibling embryos; transcript accumulation is actually elevated in mutant kernels at 14 dap and 16 dap. Moreover, transcription of several heat shock protein genes are also up-regulated in 14 dap and 16 dap mutant kernels. Our data suggest a central role for EMP2 during negative regulation of heat shock transcription, and supports a model in which the development of emp2 mutant embryos is arrested due to an un-attenuated heat shock response. Furthermore, this model implies that mutant kernels accumulate aberrant, non-functional EMP2 transcripts. We are currently testing this model.

P34 Laminate coleoptile (lco): a study in organ identity
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In recent years our laboraotry has developed a system for studying organogenesis using the coleoptile as a model. This juvenile organ is derived from the surface of the scutellum from non-shoot meristemic tissue. The coleoptile forms an ensheathing tube which protects the juvenile leaves as they push through the soil and provides support to the small plant. The fused coleoptile has two lateral veins and, when mature, splits in a predictable manner revealing the growing leaves. Coleoptiles are screened in the greenhouse, thus making it possible to recover defective mutants that would otherwise not germinate in the field. We have identified several mutants that affect coleoptile organogensis. One such mutant isolated from EMS mutagenized material, laminate coleoptile, has a dramatic morphological change resulting in multiple veins, a lack of organ fusion, and increasingly leaf-like properties towards the margins. Close examination of the margins revealed that this area contains blade and sheath-like regions with a ligule/auricle-like boundary. Such morphological changes raise questions regarding the disputed homology of the maize coleoptile and the leaf. Morphology of the subsequent shoot, leaves, and flowers is also altered. Here we describe the morphological features of laminate coleoptile, our SSR mapping results, and discuss the implications of this homeotic mutation.

P35 Wandering carpel- a new mutation that alters symmetry of maize flowers
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Although most monocots have radially symmetric flowers, this taxon includes orchids, which have spectacular, strongly zygomorphic flowers. Symmetry in grass flowers has attracted far less attention, probably a consequence of their lack of petals; nonetheless, it is clear that the tendency within the group is toward zygomorphy. Maize flowers are strongly zygomorphic, having two lodicules, rather than three, a pistil formed of two indeterminate, sterile carpels, which form the silk, and a third, determinate, fertile carpel, and a single ovule rather than three. We have found and characterized a new mutation, Wandering carpel (Wcr), which alters the symmetry of the flower, both by conversion of individual flowers to a radial symmetry and/or by altering the orientation of the flower on the inflorescence. Mature ears show kernels with aberrant orientation in which the embryo is not restricted to the anterior (closer to the tip of the ear) face of the kernel but can be found on any side. Wcr is a semidominant mutation and maps to 1S. Wcr shows maternal inheritance, partial penetrance, and background dependence for strength of expression. Even as a homozygote in W23, where it is most strongly expressed, approximately 40% of flowers/kernels on an ear are normal. In addition to obliquely or reverse-oriented flowers, SEM studies show, at a much lower frequency, flowers with extra carpels and development of the lower floret that is normally suppressed. We hypothesize that WCR is involved in the formation of a growth inhibitor, which is asymmetrically distributed in the floral meristem.

P36 Developmental expression of protease activity in CMS-S and normal maize pollen
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Cytoplasmic male sterility (CMS), which is the maternally inherited failure to produce functional pollen, results from the expression of novel, chimeric genes in the mitochondria. Based upon the role of mitochondria in signaling programmed cell death (PCD) in vertebrates (Green and Reed, Science 281:1309), we propose that CMS pollen collapse results from a PCD pathway that involves the induction/action of a protease. In CMS-S maize, the expression of reproductive failure occurs in developing male gametophytes. We used activity gels to study protease activities. In order to gather knowledge on protease activity during pollen development, we collected developing pollen samples of the CMS-S line, a normal cytoplasm line, and a CMS-S line restored to male fertility by the Rf3 allele. All materials had the Mo17 inbred nuclear background. Microspores were collected from pre-emergent tassels, collapsed or starch-filling pollen was collected from post-emergent tassels, and mature pollen was collected from dehiscent anthers. Protease activities were observed in each of the development stages. All microspores had an activity with an apparent molecular weight of 55kD. Starch-filling and mature normal-cytoplasm pollen had activity with an apparent molecular weight of 60kD. Collapsed CMS-S pollen and mature CMS-S Rf3 pollen had a protease activity with an apparent molecular weight of 50kD. Thus the 50 kD activity was unique to S cytoplasm and its expression was delayed in the presence of the Rf3 allele. The protease class of each activity band is currently under investigation. The 50 kD activity is a candidate executioner protease in the pathway leading to CMS-S pollen collapse.

P37 Dorsoventral patterning of the maize leaf
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The first steps in lateral organ formation include the recruitment of founder cells from the meristem and the establishment of new developmental axes relative to the main body axis. Characterization of the leafbladeless1(lbl1) mutant phenotype suggested that lbl1 is required to establish adaxial cell identity in leaves and leaf-like lateral organs. In the absence of LBL1 activity, cells obtain an abaxial identity that results in the formation of radially symmetric, abaxialized leaves. Less severe leaf phenotypes include the formation of ectopic laminar outgrowths at the boundaries of abaxialized sectors on the adaxial leaf surface, and the bifurcation of leaves. The semi-dominant Rolled1-O (Rld1-O) mutant of maize also affects dorsoventral patterning in that the polarity of the leaf is inverted. Double mutants between Rld1-O and lbl1 resulted in a mutual suppression of both phenotypes. These results suggest that lbl1 and rld1 act in an opposing fashion on the same pathway, or that lbl1 and rld1 negatively regulate each other. In order to further characterize the lbl1, Rld1 and lbl1;Rld1 double mutant phenotypes, we isolated the maize homologs of the Arabidopsis Yabby genes which are expressed in the incipient primordium and in the abaxial domain of developing leaves and floral organs. We are currently in the process of analyzing their expression patterns in wild type and in the different dorsoventral patterning mutants in maize. Preliminary data suggest that the yabby expression pattern is different in maize. As in Arabidopsis, the maize yabby genes are expressed throughout the incipient primordium, but interestingly, expression later in development becomes restricted to the adaxial side and to the margins of the leaf.

P38 Characterization of cell division patterns in the xcl (extra cell layers) mutation.
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During leaf initiation and development, cells divide in either the anticlinal plane (perpendicular to the surface of the organ) or in the periclinal plane (parallel to the surface of the organ). Cell divisions in the protoderm occur in the anticlinal plane in order to expand the surface of the developing leaf. Due to its extremely regular patterns of cell division and differentiation, the maize leaf provides an excellent system for identifying mutations which affect cell division patterns. The xcl (extra cell layers) mutation leads to the formation of additional cell layers below both the adaxial and abaxial epidermis of leaf blade and sheath without affecting the arrangement of mesophyll and bundle sheath cells. The origin of the extra cell layers has been determined using clonal analysis with anthocyanin markers and by examining cell division patterns in the meristem and developing leaves. The extra cells appear to arise from aberrant periclinal divisions in the protoderm and have epidermal features, indicating that they differentiate according to lineage and not position. The presence of the extra cells also affects the differentiation of the epidermis. Cells are square rather than rectangular, less crenulated, and stomatal frequency is decreased. We have examined cell division planes in the epidermis of developing leaves using nail polish replicas in order to determine the effects of xcl on epidermal development. While overall plant morphology is not severely affected by the xcl mutation, xcl leaf blades can be twice as thick but only half as wide as wild-type leaves. Leaf sheaths are also narrow, with extra cell layers predominantly under the abaxial epidermis. The effects of xcl on leaf morphology in different genetic backgrounds will also be presented.

P39 A pair of putative fie genes and it parent-of-origin dependant allelic expression in maize
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A group of genes (medea, fie, fis2) that play a very important role in the early stage of embryo and endosperm development have been identified as knock-out mutants in Arabidopsis. All three of them have been shown to have a parent-of-origin dependant expressional pattern. By taking advantage of the maize EST project, a search program has been used to identify putative homologs of these genes. Based on the EST information two maize genes with significant homology at the nucleotide and protein level to the arabidopsis fie gene have been cloned. Both of these loci, Zmfie1 and Zmfie2, appear to be single copy genes in the maize genome based on southern blot analysis. Northern blot analysis showed that they are both expressed during endosperm development. One of them (Zmfie1) exhibits allele-specific expression dependent on the parental origin, indicating genomic imprinting; the maternal allele is expressed while its paternal allele is silent in the early stage of embryo and endosperm development.

P40 Mutants with reduced seed expression have multiple phenotypes and identify two new regulators of the maize anthocyanin pathway

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To better understand the processes involved in the tissue-specific regulation of gene expression, a mutant screen was done in our lab with the aim of identifying new factors involved in the maize anthocyanin pathway. Mutagenesis was carried out using B-Peru, one of two b1 alleles that confer anthocyanin pigmentation in the aleurone. We screened for mutants that showed reduced seed color relative to wild type. After screening out those mutants that are allelic to previously characterized regulatory or biosynthetic genes of the pathway and those mutants with an unstable phenotype, we are continuing to work with 40 mutants that appear to represent mutations in novel genes. These mutants are designated pac for pale aleurone color. Many of these mutants show profound developmental defects. We are using RNase protection assays to identify those mutants that have reduced expression of either the regulators, b1, c1, and pac1 or the biosynthetic genes of the anthocyanin biosynthesis pathway. Two mutants are of particular interest based on these experiments. pac5 shows reduced expression of B-Peru and all three biosynthetic genes we tested. The pac5 mutant is pleiotropic; plants homozygous for pac5 show numerous defects in both vegetative and reproductive tissues. pac7 shows some reduction of the expression of c1 and a more severe reduction of the biosynthetic gene a1. Plants homozygous for pac7 are phenotypically normal, expect for their lack of seed pigment. Although pac7 maps to 3L near the known regulator vp1, allelism tests indicate that it is not allelic to vp1 but instead represents a novel regulator of the pathway.

P41 Genotypes and Genes for Improved Methods of Maize Transformation

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The most widely used method for producing transgenic maize targets the totipotent callus tissue derived from the scutellum of immature embryos. This method is limited to a few genotypes capable of forming totipotent callus from that source. Improved methods could be developed if we acquired a basic understanding of totipotency in maize. Our objectives are: 1) determine the genetic positions of genes controlling totipotency, 2) use differential libraries to isolate genes expressed in responsive (e.g. H99, A188, HiIIA and HiIIB) and recalcitrant genotypes (e.g. Mo17 and B73), 3) identify genes and processes involved in callus initiation, 4) identify genes and mechanisms that control callus initiation and 5) develop approaches to improve methods of maize transformation. A population of 185 recombinant inbred lines (RILs) of Mo17 x H99 has been surveyed for callus initiation in two consecutive years. Genes associated with callus initiation in that population have been located to chromosomes 3, 5, 8 and 9. The response of several RILs equaled or exceeded that of H99 in both years. A subset of the responsive RILs have been transformed by particle bombardment.

P42 A combinatorial approach to assembling the liguleless3 genetic network
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Leaf development is orchestrated, in part, by the knotted1-like homeobox (knox) genes. The protein products of these genes are thought to be DNA binding proteins and are presumably involved in transcriptional regulation. Past work has led us to propose that the knox gene liguleless3 helps determine regional identity along the proximo-distal axis in a temporal not spatial manner. To better understand this phenomenon at the molecular level, we initiated a yeast screen to identify genes directly regulated by LG3. Through our screen, we were able to identify a putative binding sequence of LG3 that is also conserved in the second intron of three maize knox genes including liguleless3 itself. This same binding site is conserved both in position and in sequence in the knox rice orthologs. This conservation in sequence and position between maize and rice prompted us to search the Syngenta genomic rice database for other genes that contained the LG3 binding site. Several rice genes were identified that contained the motif within potential regulatory regions and the maize orthologs were subsequently tested for altered expression patterns in Liguleless3 mutants. One of the genes, osk2, showed down regulation in developing leaves of a Lg3 mutant when compared to wildtype siblings. Experiments are in progress to determine whether or not this kinase is a bona fide target of LG3. (This work was done in collaboration with researchers at Syngenta.)

P43 Cloning and molecular characterization of bif2
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barren inflorescence2 (bif2) plays a critical role in axillary meristem formation during maize reproductive development. In bif2 mutants, axillary meristems do not initiate resulting in the production of fewer ear shoots, tassel branches, spikelets, florets and floral organs in the tassel and ear. RNA in-situ hybridization with kn1 shows that the defect occurs early in development as axillary meristem initials fail to maintain kn1 expression. We have cloned bif2 using traditional transposon tagging. A Mu1 element was identified segregating within 1 cM of the bif2 mutant phenotype in allele bif2-47330 and flanking DNA was cloned by constructing a subgenomic library. Confirmation that bif2 has been cloned was obtained by PCR and sequence analysis of an additional allele, bif2-1504, which has a Mu1 element inserted 20 bp away from the insertion site of the Mu1 element in bif2-47330. Southern analysis does not reveal a polymorphism in alleles bif2-1606, bif2-70, bif2-77 and bif2-N2354. RNA in-situ hybridization shows that bif2 is expressed in axillary meristems. We propose a model for the role of bif2 and kn1 in axillary meristem intitation.

P44 Molecular Genetic Approaches to Identify Glossy15 Target Genes
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The first five-to-six juvenile leaves in maize differ from the upper adult leaves in the expression of a large set of epidermal cell characteristics. These traits include leaf waxes, the differentiation of bulliform cells and leaf hairs, cell shape, and cell wall biochemistry. Phenotypic analysis of glossy15 (gl15) mutations has demonstrated that the Gl15 gene coordinately regulates the expression of each of the traits that define juvenile and adult leaf epidermal cell identity. The Gl15 gene encodes an APETALA2-like DNA binding protein whose expression appears limited to juvenile leaves. Thus, GL15 presumably regulates leaf epidermal cell differentiation through the transcriptional activation and/or repression of target genes that mediate changes in the composition of leaf waxes and the cell wall as well as cellular morphology. Presented here is an update of efforts to define candidate target genes of GL15 activity that program the differentiation of juvenile and adult leaf epidermal cells. Genetic screens have identified mutations that specifically affect wax composition (the existing collection of glossy mutants), macrohair formation (macrohairless, mhl), and epidermal cell wall biochemistry (ecw1). These mutations represent defects in the expression of a subset of phase-specific epidermal phenotypes, which demonstrate that GL15 regulates target genes in at least three genetically distinct cellular processes (wax production, hair formation, cell wall biosynthesis). The phenotypes associated with some of these mutations suggest that Gl15 may function primarily as a repressor of adult epidermal cell differentiation. Double mutant analysis of glossy, mhl, and ecw1 mutants in combination with gl15 have revealed that most of these mutations are epistatic to gl15, providing genetic evidence that they indeed act downstream of Gl15. Progress on the molecular cloning of candidate Gl15 target genes by transposon tagging and expression profiling approaches, as well as modifying GL15 activity in transgenic maize will also be discussed.

P45 Hunting for gene hierarchies regulating endosperm development: an effort based on forward and reverse genetics, functional genomics and proteomics.
Olsen , Odd-Arne (1); Lid, Stein Erik (1); Meeley, Robert (2); Shen, Bo (2); Tarczynski, Mitchell (2); Chamberlin, Mark (2); Niu, Xiaomu (2); Lawrence, Jim (2); Morgante, Michele (3); Hanafey, Mike (3); Jung , Rudolf (2); Nichols, Scott (2) (1) Agricultural University of Norway, Ås, Norway; (2) Pioneer Hi-Bred International, Johston, IA,; (3) DuPont Agricultural Products, Wilmington, DE.; (4)

In the Pioneer TUSC population of corn Mu-insertion lines, 12.480 (30%) of the F2-ears segregate for incomplete grain filling. Mutant seeds from these lines have been sectioned for light microscopy examination. Currently, all mutant endosperm phenotypes described in the literature, plus several novel phenotypes have been discovered, including missing aleurone (dek1), two layers of aleurone, multiple layers of aleurone, unorganized aleurone layers, defective aleurone cells, defective starchy endosperm, unorganized transfer cell layers as well as missing transfer cell layers. Efforts are currently underway to clone selected mutant genes using Mu-tagging. In this effort, co-seg analysis is carried out using known Mu-elements as probes in backcrossed families segregating for the mutant phenotype. Verification of candidate genes is sought through identification of additional insertion alleles using the TUSC reverse genetics facility. Longer versions of candidate genes are identified in the Pioneer/DuPont EST collection, and information on transcript and protein expression is obtained using massively parallel signature sequence (MPSS, Lynx Technologies) and 2D/Mass spech technologies (Oxford Glyco Sciences). The use of this approach to endosperm development is illustrated by our effort to clone the dek1 gene using a novel dek1 allele identified in the TUSC microscopy screen.

P46 Characterization of extended auricle (eta) a leaf developmental mutant that affects the blade/sheath boundary in maize

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One of the major questions in development is how cell fate is determined and how patterns are established. The maize leaf provides an elegant and tractable model for examining cell fate determination and pattern formation. Although the ligule has been well-characterized developmentally, auricle development has been largely ignored. We have identified a recessive, EMS-induced mutant that we call extended auricle (eta). Mutant eta individuals have a wavy overgrowth of auricle tissue and the auricle/blade boundary is diffuse. The mutant phenotype can be detected at the seedling stage, but becomes more pronounced as the plants mature. The eta phenotype is largely background dependent, being more severe in Mo17 and less pronounced in B73. RT-PCR analysis indicates that the eta phenotype is not a result of ectopic knox gene expression. The eta mutation has been mapped to the long arm of chromosome five using TBs and has been mapped to a bin using SSRs. Further molecular and genetic analyses will help us to understand the role of eta in maize leaf development, and specifically in formation of the blade/sheath boundary.

P47 Developmental Regulation of an Early Nodulin-like Gene in Maize Endosperm

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A maize homologue of soybean GmENOD93 (Early Nodulin gene) was isolated from the maize inbred line Bz-McC(W22). This gene(ZmENOD) is present in a single copy in the maize genome and is located chromosome 9, as shown by OMAL(oat-maize addition line). ZmENOD is abundantly expressed in the endosperm starting 2 weeks after pollination. Expression level peaks at 4 weeks, after which time it decreases gradually. This gene is also expressed in very early developmental stages of shoots and roots, 4 days after germination. After that, the transcript level decreases gradually. ZmENOD mRNA is not present in seedling at all, but the transcript level is dramatically increased at bottom parts of shoots one hour after cutting. ZmENOD may play role in early developmental stages of endosperm, shoots, and roots. The possible physiological function and the structure of ZmENOD gene will be discussed

P48 Characterization of Maize stk1

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stk1 is the proximalmost of the 10 genes in the 33-kb bz gene-rich region of 9S. To characterize this gene, a cDNA was isolated from a mixed tassel cDNA library. An apparently full length stk1 cDNA was obtained. It is 2,859bp long and contains a 197bp 5,-UTR,, a 288bp 3,-UTR, and two introns. Conceptual translation of the stk1 cDNA produces a 790-amino-acid protein, which has high homology to serine/threonine protein kinases in the 3rd exon. This gene is highly expressed only in maturing tassels and not expressed at all in other tissues examined. The stk1 transcript is only detectable in wild type Bz-McC, but not detectable in the sh-bz-X2 deletion mutant, in which the entire bz gene rich region is deleted. Transmission and expression data suggest that stk1 may play a role in normal pollen development in maize.

P49 Cloning of narrow sheath2, a duplicate factor gene required for recruitment of a lateral domain in the maize leaf.

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TALK #21 ABSTRACT

THE GENE

SUNDAY MORNING

P50 Using cell pattern mutants to explore the function of endoreduplication during leaf development.
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Endoreduplication occurs when DNA synthesis continues in the absence of mitosis. In plants, endoreduplication often coincides with cell expansion and differentiation. The precise function is not well understood, although a high proportion of leaf cells from dicots contain endoreduplicated nuclei. Genetic evidence suggests that endoreduplication may signal cells to enter a particular differentiation pathway, such as trichome development in Arabidopsis (Traas et al. 1998). Endoreduplication is also tightly linked to cell fate decisions during Drosophila embryogenesis (e.g. Lilly & Spradling 1996). An alternate hypothesis is that endoreduplication is in response to a differentiation process that has already begun. In this case, the function of endoreduplication could be to mediate cell size in a developing leaf, thereby maintaining tissue integrity for interconnected cells that are growing differentially. To distinguish these hypotheses, we are making use of a class of cell pattern mutants that show stochastic endoreduplication associated with excessive cell expansion. The mutants rli1-wty and chaos1-0 exhibit arrested mitosis and endoreduplication as well as occasional abnormal differentiation of epidermal cells. If differentiation is signaled by endoreduplication, we expect that a proportion of abnormally differentiating cells in mutant leaves will correspond to a similar proportion of abnormally endoreduplicating cells during early development. We first characterize the extent and location of endoreduplication in normal maize leaves. Flow cytometry demonstrates that a proportion of nuclei in both normal and maize leaves have elevated DNA content (Cocclione & Sylvester, obs.). To identify endoreduplicated cells in the intact maize leaf, we use an epifluorescence microscope equipped with a cryogenically cooled CCD camera and filters to detect emission from the DNA-specific fluorochromes DAPI and propidium iodide. Quantitative fluorescence reveals endoreduplication in a variety of normal leaf epidermal cells, including developing hairs and intercostal cells. The developmental profile of mutants indicates an early onset of endoreduplication in unexpected cell types. The results will provide a necessary framework for continued analysis of endoreduplication during maize leaf development.

P51 Toward a function for rough sheath2 on the cellular level
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The rough sheath2(rs2) gene is essential for wild type leaf development and has been proposed to be a regulator of certain knox (KN1 related homeobox) genes. rs2 belongs to a distinct subfamily of myb related genes which include the Antirhinum gene phantastica and the Arabidopsis gene asymmetric leaf1, both of which are important to leaf development. Members of this family contain a highly diverged myb domain in the N-terminal portion of the protein and a large conserved C-terminal domain of unknown function. Preliminary experiments to identify an RS2 binding site suggested that RS2 has a low affinity for dsDNA. To address the possibility that a cofactor is required for DNA binding, a yeast two-hybrid screen was used to identify proteins that interact with RS2 at the cellular level. The protein recovered most frequently from the screen was RS2 itself, suggesting that RS2 forms homodimers. The domain necessary for homodimerization was mapped to a portion of the conserved C-terminal domain. In addition to RS2 several other proteins were identified and are being further characterized.

P52 Bilateral coleoptile, midrib or prophyll?
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In 1999 I observed a mutation in which structures were growing from coleoptile tissue at the tip of the coleoptile. Initially the tip of the coleoptile had an arrowhead appearance. If the seedlings are allowed to grow for a longer period of time two long, colorless, hornlike, structures grow from the coleoptile. In other seedlings the coleoptile is thickened in a bilateral fashion. Initially I reported this mutant as „rooty coleoptile‰. But following histological examination and due to the consistent bilateral symmetry these structures are clearly leaf tissue. In cross-section the thickened coleoptile bears a strong resemblance to a prophyll. The hornlike structures appear to be the same structure, but unattached over the length of the coleoptile. If in fact these structures are analogous to prophyll this would have bearing on the origins of the coleoptile and perhaps the nature of the scutellum. In 1960 Sidhu and Everett (MNL 60:25) described a mutant they called hornlike coleoptile (hc). They also mention a mutant called by bikeeled. Their description sounds quite similar to the current mutant. Given the variable phenotype and that we do not know the actual origin of the structure I suggest this mutant be given a descriptive name bilateral coleoptile1 (blc1). blc1 was observed in selfed progeny from the population [Cacahuacintle Dulce x Temperate Sweet]. In 2000 blc1 appeared to be dominant in reciprocal crosses.

P53 A recessive allele of *rs1* suppresses the *rs2* mutant phenotype.
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Recessive mutations in the gene *rough sheath2* (*rs2*) have a phenotype similar to but more severe than dominant *rough sheath1* (*rs1*) alleles. Dominant *rs1* alleles disturb the blade-sheath boundary and cause proliferation of sheath like tissue within the auricle and at the base of the blade.[1] In addition to these phenotypes, *rs2* mutants are reduced in stature and have abnormalities in both the ear and tassel. In the ear extra silks (the elongated styles of maize flowers) form[2] and the kernels have a reversed orientation; in the tassel there is excessive branching and aberrant development of male flowers may also occur. In *rs2* mutant alleles, expression of *knox* gene products in leaves has been demonstrated and is convincingly shown to occur for *rs1*.[2-4] A decrease in auxin polar transport occurs as well.[5]

We wished to determine what contribution ectopic expression from *rs1* makes to the *rs2* mutant phenotype. To this end, a putative knockout allele of *rs1* (*rs1-872*) that has a *Mutator* element inserted into the second exon was crossed to *rs2*. The *rs1-872* allele was obtained from material generously provided by Bob Meeley and Pioneer's Trait Utility System for Corn. This allele alone has no apparent homozygous phenotype; however, it does partially suppress the *rs2* mutant phenotype. We are investigating whether the residual phenotype seen in *rs2*; *rs1-872* mutant plants is due to gene product from the *rs1-872* allele or to ectopic expression from other *knox* genes.

1 Becraft, P.W. and Freeling, M. (1994) *Genetics* 136, 295-311.
2 Schneeberger, R., Tsiantis, M., Freeling, M. and Langdale, J.A. (1998) *Development* 125, 2857-65
3 Timmermans, M.C., Hudson, A., Becraft, P.W. and Nelson, T. (1999) *Science* 284, 151-3
4 Tsiantis, M. et al. (1999) *Science* 284, 154-6.
5 Tsiantis, M., Brown, M.I.N., Skibinski, G. and Langdale, J.A. (1999) *Plant Physiology (Rockville)* 121, 1163-1168.

P54 The Interactive Maize Plant (*IMP*)

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An overview of the proposed contents of the Interactive Maize Plant (*IMP*) is presented. The purpose of *IMP* is to develop and extend the considerable educational facility that is inherent in MaizeDB for people in developing and developed parts of the world who are interested in maize - anyone who has access to the internet. *IMP* will cater for a wide range of users - from K12 scholars and teachers to undergraduates, graduates and new and not so new researchers of maize and the general public. *IMP* comprises image maps of the maize plant linked to attractive and botanically accurate and annotated line-drawings and images of associated macro- and micro-morphology, anatomy, developmental stages etc. of maize. All of this will be based on an Ontology with its own appropriate Controlled Vocabulary of terms. Other facilities provided are: Interesting Factts, *Zea mays* - A Scientific Fact Sheet; FAQs; Glossary & Synonym facility. Definitely a work in progress. Access *IMP* via **http://www.agron.missouri.edu/**.

P55 Protein expression of the maize bZIP protein LIGULELESS2

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liguleless2-R (lg2-R) is a null mutation that affects the formation of the ligule-auricle region of the maize leaf. lg2-R mutants lack ligule and auricle on the lower most leaves of the plant. On higher leaves, ligule and auricle begin to appear at the leaf margins and with each successive leaf the phenotype gradually becomes less severe. Where the ligule and/or auricle is lacking, the sheath can extend into the blade. lg2-R mutants also have phenotypes associated with the vegetative to reproductive transition. Past work identified the lg2 gene by transposon tagging and found it to encode a bZIP protein. Antibodies to a unique region of the LG2 protein were produced in rabbits. Westerns indicate the LG2 protein accumulates in developing leaves at the preligule band and ligule ridge stages. The LG2 antibody detects no protein in developing leaves of plants homozygous for either a deletion allele or the reference allele. Immunolocalizations detect LG2 protein accumulation in developing ligules. Interestingly, accumulation is higher in the region of the developing ligule that is oppressed to the leaf.

P56 IDENTIFICATION OF PROTEINS INVOLVED IN ZEIN mRNA TRAFFICKING IN Zea mays.
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The endosperm provides the primary source of nutrition for maize seedlings following germination. During endosperm development protein bodies, composed of zeins, form within the endosperm. Zeins serve as the primary source of protein for the seedling during germination. Given that endosperm cells are large (100µm), it is hypothesized that zein mRNAs are actively transported to their site of translation, possibly along actin microfilaments. The translation factor EF1A and microfilaments have been previously shown to surround endoplasmic reticulum regions at sites where protein bodies are forming. Work has begun to isolate and identify proteins that bind to the 3' untranslated region (UTR) of the 27-kDa gamma-zein mRNA. Extracts of developing maize kernels were subjected to several types of chromatographic separation and the fractions were tested for their ability to bind and alter the mobility of a 299 base RNA probe derived from the 3' UTR. Additional experiments were performed that identified proteins transferred to nitrocellulose that bind to this probe. At least 5 polypeptides have been shown to bind to the 3' UTR probe in vitro. One of these proteins was shown by MALDI-TOF mass fingerprinting and tandem mass spectrometry (MS-MS) to be eukaryotic initiation factor 5A (eIF-5A). Work is now being done to identify a gamma-zein mRNA-binding protein of approximately 21-kDa (p21). p21 has been shown to bind to and alter the mobility of the 3' UTR probe in gel shifting. p21 has been purified to ~50% purity by reversed-phase HPLC and is currently being analyzed by MALDI-TOF and MS-MS. Also, a protein of approximately 28-kDa (p28) has been purified to ~90% purity and has been shown by LC/MS/MS to be nascent associated-polypeptide complex aalpha chain. Following the identification of p21, work will be done to determine its RNA binding specificity and interactions with other proteins.

P57 Grh2 and Grh4 interaction expressing strong resistance to leafhopper species in rice
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Nephotettix cincticeps Uhler (Green rice leafhopper: GRH) is an insect pest in temperate Asian rice field. Four GRH resistance genes (Grh1, Grh2, Grh3, and Grh4), that will be concerned with sucking inhibition, have been identified throgh RFLP mapping. In this study, we developed near-isogenic lines (NILs) for Grh2 and Grh4 from the cross between susceptible Japonica variety Kinmaze and resistant Indica variety DV85 with the aid of molecular markers. The resistance evaluation of the NILs was carried out through antibiosis tests against two kinds of leafhopper species, N. cincticeps and N. virescence, those were serious vectors for several virus disease in temperate and tropical area, respectively. Nymph mortality of the NILs at 3 days after infestation was compared with resistant and susceptible checks. NILs carrying one of the resistance genes only showed weak resistance or susceptibility. On the other hand, NILs carrying both of the resistance genes, Grh2 and Grh4 expressed strong resistance as the same level with resistant variety DV85. The results clarified Grh2 and Grh4 interaction expresses strong resistance to two leafhopper species in rice.

P58 The Role of Glk Genes in Photosynthetic Development

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Mesophyll and bundle sheath cells of C4 maize leaf blades develop chloroplasts that differ in their biochemistry and morphology. The Golden 2 (G2) gene is required for this tissue-specific differentiation. Recessive g2 mutants have defective chloroplasts in C4 bundle sheath cells but develop normal chloroplasts in C4 mesophyll cells. Expression patterns obtained from Northern analysis revealed that G2 is primarily expressed in C4 bundle sheath cells whereas a Golden 2-like (Glk) gene (ZmGlk1) is expressed predominantly in C4 mesophyll cells. This profile suggests that while G2 regulates chloroplast differentiation specifically in C4 bundle sheath cells, ZmGlk1 may promote differentiation of C4 mesophyll cells. Both G2 and ZmGlk1 are proposed to encode transcription factors belonging to the ŒGARP, superfamily. Yeast GAL4 trans-activation assays have shown that the amino-terminal portion of both proteins is capable of trans-activating a reporter gene and yeast-two-hybrid experiments revealed that the carboxyl-terminus may be involved in homo- and hetero-dimerization. Glk genes have also been identified in the C3 grass rice. Data on amino acid sequence identity, intron position and chromosomal location suggest that OsGlk1 is orthologous to ZmGlk1 and OsGlk2 is orthlogous to G2. The overlapping expression patterns of the two rice Glk genes suggest that they act redundantly in C3 plants. Thus, it is possible that the acquisition of specialized function by Glk genes was one of the key points during the evolution of C4 photosynthesis.

P59 Arabidopsis Homologs of Drosophila Trithorax Genes. Structure and Evolution of the SET-Domain.

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Genes belonging to two major families of antagonistically acting regulators, the Trithorax-Group (Trx-G) and the Polycomb-group (Pc-G), are implicated in the establishment and maintenance of appropriate expression patterns during development and epigenetic regulation. Plant homologs to the Trithorax family genes have not been characterized yet. Using the most highly conserved motif, the SET domain found in proteins belonging to families of both repressors and activators, we have studied the evolution of the SET domain within the Trithorax family of proteins. Two Arabidopsis homologs of Drosophila trithorax genes have been characterized. Their molecular structure and expression patterns have been established. A new structural element has been discovered. Its presence in animal and plant genes, while absent from yeast, suggested that it has appeared later in evolution, probably as a function related to multicellularity. The co-evolution of this new motif with the evolution of the SET-domain makes it a signature feature for the Trithorax family. The molecular architecture of the different members of the Trithorax family allows some predictions regarding their function to be made.

P60 Insertion-Deletion Polymorphisms in 3, Regions of Maize Genes Occur Frequently and Can Be Used as Highly Informative Genetic Markers

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Single nucleotide polymorphisms (SNPs) are the most frequent variations in the genome of any organism. SNP discovery approaches like re-sequencing or data mining enable the identification of insertion-deletion (indel) polymorphisms. These indels can be treated as biallelic markers and can be utilized for genetic mapping and diagnostics. In this study 655 indels have been identified by resequencing 502 maize loci across 8 maize inbreds (selected for their high allelic variation). Out of these 502 loci, 433 were polymorphic, with indels identified in 215 loci. Of the 655 indels identified, single nucleotide indels accounted more than half (54.8%) followed by two and three nucleotide indels. Three novel miniature inverted repeat transposable element (MITE) like sequences were identified as insertions near genes. The utility of indels as genetic markers was demonstrated by using indel polymorphisms to map 7 loci in a B73 x Mo17 recombinant inbred population. The haplotype heterozygosity values were calculated for the SNPs and indels and found to be 0.57.

P61 Mapping Sexual and Apomictic Tripsacum Floral cDNAs in Zea

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Comparisons using cDNA (cloned DNA from expressed genes) from different species greatly increases our understanding and ability to identify the changes in the genetic content of related species through the process of evolution. This research utilized cDNA isolated from developmentally staged female flowers of Tripsacum, a relative of modern maize (corn), with differing modes of reproductive behaviors. The gene expression clone libraries potentially carry the gene(s) responsible for the regulation of fertility, both apomixis and sexual reproduction, in Tripsacum. The cDNAs E2-42 and M2-62, from sexual diploid Tripsacum flowers, showed monomorphic band patterns when screened in maize. The cDNA sequence L4-14, from apomictic tetraploid Tripsacum flowers, revealed polymorphic bands patterns. The L4-14 polymorphisms were mapped in the UMC Tx/CO IF2 and scored as both 1:2:1 and 3:1 segregation ratios. These scores were compared to a subset of ordered loci from the UMC Tx303 x CO159 map data panels, Maize Database, University of Missouri. Two maize map regions were identified as linked to the L4-14 locus. These regions included bin 6.05-6.08 of maize chromosome 6 and bin 8.00-8.05 of maize chromosome 8. The linkage of the L4-14 cDNA to two different maize chromosomal regions also corresponded to a known duplication region within the maize genome. Results of these analyses will be presented.

P62 The Intermated B73 x Mo17 Genetic Map: A Community Resource
Davis, G.(1); Musket, T.(1); Melia-Hancock, S.(1); Duru., N. (1); Sharopova, N.(1); Schultz, L. (1); McMullen, M.D.(1,2); Sanchez, H. (1); Schroeder, S.(1); Garcia, A.(1); Polacco, M.(1,2); Woodman, W. (3); Long, M.J. (3); Lee, M. (3); Vogel, J. (4); Wineland, R. (5); Brouwer, C. (5); Arbuckle, J. (6); Houchins, K. (1,2); Davis, D. (1); Bergstrom, D. (1); Cone, K. (1); Liscum. E. (1); and Coe, Jr., E.H. (1,2). 1) University of Missouri-Columbia; (2) USDA, ARS, PRGU; (3) Iowa State University, (4)DuPont Agricultural Biotechnology, (5) Pioneer Hi-Bred International, (6) Novartis

A high-resolution genetic map was constructed in a B73 x Mo17 population derived after four generations of random-mating. Theoretically, the random-mating process increases the number of recombination events present in an individual mapping line by 3-fold. Our data support this. One thousand low copy number restriction fragment length polymorphism (RFLP) loci and more than 700 simple sequence repeat (SSR) loci were mapped. Screening images of the RFLPs and SSRs are available at http://www.agron.missouri.edu/images.html. A table detailing the primer information for the SSRs is available at http://www.agron.missouri.edu/ssr.html. The SSR primers are derived from public EST sequence data and from enriched libraries produced in-house. The RFLPs include many markers mapped on other public populations. In addition, novel *PstI* clones and *Mu*-tagged clones have been added to the map. The map has better local order resolution than prior public maps because of the increased number of recombinants per individual. It serves as a bridge between prior public maps and the maize physical map currently under construction. Markers from the map are being assigned to BAC clones by both hybridization and PCR-based assays. A subset of lines from the mapping population has been selected for researchers who what to map a gene of interest. The lines are available from the Maize Genetics Cooperation Stock Center and will be available as DNA in microtiter plates for PCR-based assays from the Missouri Maize Project.

P63 Genome Characterization by Screening of Maize BAC Libraries
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A *Hin*dIII and an *Eco*RI maize bacterial artificial chromosome (BAC) library have been screened with complex maize probes. These libraries and a third new BAC library are being used to construct a genetically anchored maize physical map. All of the libraries were made from inbred B73. The *Hin*dIII library made at the Clemson University Genomics Institute (CUGI) has an average insert size of 135 Kb with a genome coverage of 10X. The *Eco*RI library made at the Childrens Hospital Oakland Research Institute has an average insert size of 160 Kb with a genome coverage of 6X. High density filter sets from the *Hin*dIII and *Eco*RI libraries containing 5X and 6X genome equivalents respectively were screened with two sets of probes. Set I is comprised of complex probes which will provide information about chromosome architecture and organellar DNA content. Probes in this set include 185bp knob repeat, ribosomal fragment, two telomere associated sequences, three centromere repeat sequences, maize mitochondrial DNA, and a chloroplast DNA cocktail. Set II contains the maize core markers. These markers have been used extensively in RFLP based mapping experiments and they correspond to the maize bin boundaries. They have been mapped on the IBM genetic map that is being used to genetically anchor the maize physical map. Probes were hybridized using standard BAC filter protocols and exposed to x-ray film after washing. Films were digitized on a large-bed scanner. Positive clones were called using HDFR 1.0. This information will be integrated with *Hin*dIII fingerprint data generated at CUGI to aid in physical map construction.

P64 Investigating the Evolution of Novel Regulatory Functions in Plants
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Transcription factors containing the Myb-homologous DNA binding domain are widely distributed in eukaryotes. The expansion of the plant R2R3 Myb gene family is likely to have occurred in conjunction with the evolution of new cellular functions, providing plasticity for plant metabolism and development. One group of R2R3 Myb genes that amplified recently (past 50 million years) in the grasses is characterized by the change of a conserved proline residue to alanine in the linker region between R2 and R3 Myb repeats (P-to-A clade). The maize P-to-A clade is characterized by at least 10 members, including the well characterized P gene, which controls a subset of flavonoid biosynthetic genes, a regulatory function probably acquired recently in the evolution of plants. So far, a single rice gene containing the P-to-A substitution has been found, further supporting the ancient origin, yet recent expansion of this group. Since the recent amplification of the P-to-A clade does not appear to be a direct consequence of the duplication of the maize genome, the study of the P-to-A group of R2R3 Myb genes provides a unique opportunity to understand the mechanism by which large gene families increase in size, and the role of amplification and divergence of regulatory genes in the development of novel cellular functions. A gain of function approach in Black Mexican Sweet (BMS) is being utilized to determine the function of the members of the P-to-A clade. In addition, the analysis of the complete sequences of some of the P-to-A clade genes are providing significant information on the possible mechanisms that may have been associated with these recent gene duplications.

P65 The highly recombinogenic bz locus lies in an unusually gene-rich region of the maize genome
Fu, Huihua (); Park, Wonkeun (); yan, Xianghe(); Zheng, Zhenwei(); shen, Binzhang(); Dooner, Hugo K() (1) Rutgers University, Piscataway, New Jersey 08854,USA

The bz locus exhibits the highest rate of recombination of any gene in higher plants. To investigate the possible basis of this high rate of recombination, we have analyzed the physical organization of the region around the bz locus. Two adjacent BAC clones, comprising a 240-kb contig centered around the Bz-McC allele, were isolated and 60 kb of contiguous DNA spanning the two BAC clones was sequenced. We find that the bz locus lies in an unusually gene-rich region of the maize genome. Ten genes, at least eight of which are shown to be transcribed, are contained in a 32-kb stretch of DNA that is uninterrupted by retrotransposons. We have isolated nearly full length cDNAs corresponding to the five proximal genes in the cluster . The average intertranscript distance between them is just 1 kb, revealing a surprisingly compact packaging of adjacent genes in this part of the genome. At least eleven small insertions, including several previously described miniature inverted repeat transposable elements (MITEs), were detected in the introns and 3' untranslated regions (UTR) of genes and between genes. The gene-rich region is flanked at the proximal and distal ends by retrotransposon blocks. Thus, the maize genome appears to have scattered regions of high gene density similar to those found in other plants. The unusually high rate of intragenic recombination seen in bz may be related to the very high gene density of the region.

P66 Molecular and Computational Analysis of Myb Genes from Sorghum and Maize
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The Myb family of genes exist widely in both plants and animals. In plants, R2R3 Myb genes encode a group of functionally diverse transcriptional regulator proteins characterized by two N-terminal conserved DNA-binding domains of approximately 50 amino acids with constantly spaced Tryptophan residues. Angiosperms contain large numbers of Myb genes (100). The functions of most plant Myb genes are unknown, but those studied to date encode transcriptional activator or repressor proteins that regulate diverse processes including development and secondary metabolism. We are interested in the evolution of the plant Myb gene family, especially with regard to the evolution of the elements that regulate Myb gene expression. The conserved structures of plant R2R3 Myb genes facilitate their isolation and sequencing. Analysis of inter- and intra-specific divergence of Myb genes should help to elucidate the evolutionary history of the Myb genes, their evolutionary patterns and constraints. Analysis of a limited number of maize and sorghum R2R3 Myb genes indicates that the 5' regulatory region, 5' UTR, and introns are quite divergent whereas the Myb domain coding regions are highly conserved. Expression studies and functional tests of the 5' regulatory regions of sorghum Myb genes are in progress. Our goal is to correlate regulatory sequences and expression patterns, identify conserved sequences (candidate regulatory elements), and test the functional roles of candidate elements using transgenic plant assays.

P67 MaizeDB: New accesses and new data. Try me.
Coe, Ed(1,2); Polacco, Mary(1,2); Schroeder, Steve(2); Hancock, Denis(2); Fang, Zhiwei(3); Sanchez-Villeda, Hector(2); Mutangadura, Tapuwa(2); Chen, Su-Shing(3); Vincent, Leszek(2); Kross, Heike(2) (1) ARS-USDA, Columbia MO 65211 USA; (2) Dept Agronomy, University of Missouri, Columbia, MO 65211 USA; (3) Dept Computer Engineering and Computer Science, University of Missouri, Columbia, MO 65211 USA

MaizeDB, www.agron.missouri.edu, has been an ARS service since 1991. Its goal is to provide a central resource for maize genome information, complete with linkages to external data repositories such as GenBank. Prior to the NSF Plant Genome Program, data were primarily obtained from published literature, MNL, electronic notebooks of laboratories engaged in mapping, GenBank and the Stock Center. Now, data are also extracted and integrated from high throughput projects, many with their own www sites. These sites can be accessed independently from the MaizeDB home page, along with 'what's new' in MaizeDB from those sites. MaizeDB data reside in an industry standard relational database management system, Sybase. Datatypes include maps, loci, probes, genetic stocks, agronomic traits, mutant phenotypes, and images along with documentation, including references, raw map data, QTL analyses, source laboratories, databases and persons, complete with address information. Database schema have proven suitably robust to support addition of new marker types, such as SSR (simple sequence repeats) with modest changes. New interfaces to the database have been created to provide rapid access to main data categories, including (1) browse lists, with or without map location constraints; (2) lists of sequences based on selectable library or map; (3) graphical map comparisons, inter and intra-species. Coming soon: graphical representation of the physical map, complete with anchors to the genetic map and the underlying, BAC contigs. We are developing trait and phenotype ontologies; these will be compatible with those for other species and databases. See accompanying poster (Vincent et al) for details. Funded by the USDA-ARS, the NSF and the University of Missouri.

P68 High resolution mapping of a 550 kb YAC contig spanning the *rp1* disease resistance locus
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Contiguous physical coverage of all seven members of the resistance gene family clustered at the complex *rp1* locus in the LH82 background has been obtained through the high resolution mapping of 13 YAC clones isolated using locus-specific probes in a 4-step chromosome walk. A minimum tiling path of 5 YAC clones spans approximately 550kb around the locus. Subclones carrying intact copies of each of the seven *rp1* homologues have been isolated, and their sequence is being determined. Of the *rp1*-related ORFs, several harbour deletions and insertions of various lengths. We will present first indications as to the structural organisation and gene content of this highly recombinogenic locus derived from sample sequencing of subclone libraries.

P69 Preliminary Radiation Hybrid Maps for Maize Chromosomes 2 and 9
Okagaki, Ron J.(1); Riera-Lizarazu, Oscar(2); Vales, M. Isabel(2); Kynast, Ralf G. (1); Ananiev, Evgueni V.(3); Odland, Wade E.(1); Livingston, Suzanne M.(1); Russell, Charles D.(1); Stec, Adrian O. (1); Zaia, Herika A.(1); Rines, Howard W. (1,4); Phillips, Ronald L.(1) (1) University of Minnesota, St Paul, MN 55108, USA; (2) Oregon State University, Corvallis, OR 97331; (3) Pioneer Hi-Bred International, Johnston, IA 50131, USA; (4) USDA-ARS, St Paul, MN 55108, USA

A high-resolution radiation hybrid mapping system for each individual maize chromosome is being developed based on oat-maize chromosome addition lines. Radiation hybrid mapping is a powerful alternative to conventional genetic linkage mapping in that it produces physical rather than genetic maps. Physical linkage relationships are determined by the co-segregation of markers among individual radiation hybrid lines. These lines carry maize sub-chromosomal pieces produced by irradiation of oat-maize chromosome addition line seed. We have isolated approximately 50 radiation hybrid lines each from addition lines of chromosome 2 and chromosome 9. Markers are being placed on this material; many of these markers have previously been mapped by linkage analysis and will be used to assess the reliability of our preliminary radiation hybrid maps. Questions we need to resolve at this point include: 1) How many individual radiation hybrid lines will be required to make a high resolution map (whole genome radiation hybrid maps made for animal species typically are based on 80 to 100 lines) and 2) What is the resolution of our maps? In addition to high resolution radiation hybrid maps we are also developing low resolution radiation hybrid maps based on a small number of well characterized lines. These mapping panels are better suited for high-throughput mapping. Progress is also being made on the generation of radiation hybrid maps for the remaining maize chromosomes. This material is based upon work supported by the National Science Foundation under Grant No. 9872650.

P70 Comparative sequence analysis of homeologous barley and rice BACs
Jorge , Dubcovsky (1); Wusirika, Ramakrishna(2); Phillip, SanMiguel(3); Carlos, Busso(1); L, Yan(1); Brian, Shiloff(4); Jeff, Bennetzen(2) (1) Depart. of Agronomy and Range Science, University of California, Davis, 95616 CA ,USA; (2) Dept. of Biological Sciences, Purdue University, West Lafayette, IN 47907 USA; (3) Purdue University Genomics Core, Purdue University, West Lafayette, IN 47907 USA; (4) National Center for Genome Resources (NCGR), Santa Fe, NM 87505, USA

Two homeologous BAC clones from the Nipponbare rice BAC library and Morex barley BAC library were identified by hybridization with RFLP markers UCW12, UCW16 and WG644. Comparison of the complete sequences of the rice and barley BACs revealed the presence of four conserved regions within a segment of 30-kb of the rice BAC and the complete 100-kb of the barley BAC. Further sequence analysis using GeneScan, GeneMark, GenSequer and BLAST searches of the EST databases showed that this segment includes four conserved genes. The fourth gene is duplicated in tandem in barley but not in rice. The four genes are in the same orientation in rice, but the second gene is in inverted orientation in barley. The comparison of the homeologus barley and rice sequence provided valuable information to establish the structure of these four genes. Gene structure was conserved between rice and barley and to a lesser extent with the Arabidopsis homeologous genes. No similarity was found between the rice and barley sequence outside the genes with the exception of short stretches of homology in the promoters and 3' UTRs. The larger distance between the first three genes in barley compared to rice is explained by the insertion of two retrotransposons of 5.3 and 7.7-kb between the first and second gene and of two nested BARE-1 retrotransposons between the second and third gene.

P71 SEQUENCING OF A 350-KILOBASE REGION OF MAIZE CHROMOSOME 4S ENCOMPASSING THE 22-KDA alpha-ZEIN GENE SUBFAMILY
Song, Rentao(1); Llaca, Victor(1); Messing, Joachim(1)

The major storage proteins in maize, called zeins, are synthesized exclusively in endosperm tissue during seed development. In normal maize genotypes, zeins constitute 50-60% of the total endosperm protein at maturity. Zeins are a heterologous protein family, classified in 4 groups (alpha, beta, gamma and delta). The beta, gamma and delta zeins are encoded by one or two genes, while the alpha zeins are encoded by a multigene family of more than 60 members. Based on sequence similarity, alpha zeins are divided into four subfamilies (z1A, z1B, z1C, and z1D). The apparent molecular weight of z1A, z1B and z1D members is 19 kDa and in z1C members is 22 kDa. According to long-range restriction mapping, most members of the z1C subfamily are clustered within a 200-kb region close to the RFLP marker php200275 on maize chromosome 4S. The sequencing of this region can provide a interesting model for studying gene amplification and gene expression. To isolate and characterize the region, a 8-fold cosmid library from maize inbred line BSSS53 was used to isolate clones containing 22 kDa zein genes. However, the relative small insert size and instability of cosmid clones made difficult the construction of a contiguous sequence from overlapping clones containing the entire gene cluster. By changing to a different cloning system based on bacterial artificial chromosomes (BACs) the missing information was obtained and a region of 346 kb from inbred BSSS53 sequenced. This is the largest contiguous sequence information from maize thus far.

P72 High-throughput Mapping Tools for Maize Genomics

Yang, Yongjie(1); Jiang, Chunxiao(1); Qiu, Fang (1); Schlueter, Jessica(1); Guo, Ling(1); Kortbein, Josh(1); Lee, Michael(1); Ashlock, Daniel (1); Wen, Tsui-Jung(1); Schnable, Patrick S.(1) (1) Iowa State University, Ames, Iowa 50011, USA

A team of molecular, quantitative and evolutionary geneticists and bioinformaticists has been assembled to develop novel high-throughput genetic mapping technologies. IDPs (InDel Polymorphisms) are a new class of co-dominant, allele-specific, genetic markers suitable for high-throughput analyses. The project team is identifying and developing 500 IDP markers specific to two widely used inbred lines (B73 and Mo17). These markers will be mapped via plus/minus PCR scoring of the 350 IBM RIs. The Mapping Array is a novel chip-based technology designed to genetically map a large number of non-redundant, sequence-defined cDNAs. During Phase I, an existing nylon-based protocol is being adapted to DNA "chips"; hybridization conditions optimized; various sources of "target" sequences (e.g., 3' UTRs, exons, full-length cDNAs and RFLP markers) tested; experimental design parameters determined; and mapping software developed. Upon successful completion of Phase I, 10,000 ESTs will be genetically mapped (Phase II). The sequences of the 3' UTRs of ESTs are of great value in distinguishing members of gene families, as potential sources of IDPs and for producing the gene-specific probes needed for the Mapping Array. The project team will sequence the 3' ends of 20,000 of the ESTs from a normalized B73 cDNA library they are preparing.

P73 Long microsatellites in corn genome

Ananiev, Evgueni(1); Lorentzen, Jen(1) (1) Pioneer Hi-Bred International, Inc. Johnston IA

see Abstract# W16

Maize Genome Workshop

Saturday afternoon

P74 Translational regulation in response to abiotic stresses is regulated by multiple signal transduction pathways

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mRNA translation becomes a more selective process following abiotic stresses, most likely due to changes in the protein synthesis machinery. Two dimensional gel systems, in vivo labeling with [32P]-Pi, and specific antisera were used to examine changes in phosphorylation of eukaryotic initiation factors (eIF) and ribosomal proteins (rp) involved in the recruitment of mRNA for translation. The phosphorylation status of eIF4E, eIF4A and eIF4B is altered by anoxia in root tips of maize. rpS6 is present in a non-phosphorylated and five increasingly phosphorylated forms under aerobic conditions and is dephosphorylated in response to anoxia and heat shock but not in response to cold or salt stress. Analysis of polysome profile of cell extracts revealed that initiation of translation is significantly reduced in response to anoxia, heat shock, cold and chemicals that modulate cytosolic calcium and pH or block phosphoinositol signaling. In some cases these chemical treatments mimicked the effect of anoxia on protein phosphorylation but in other cases they did not. We will present evidence that multiple signal transduction pathways regulate the phosphorylation status of the protein synthesis machinery and mRNA translation. Research funded by the USDA/NRI Competitive Grants Program (97-35100-4191).

P75 Photosynthetic Mutant Search(PMS): A genetic resrouce that is tailored for studies of chloroplast biogenesis.

Alice Barkan University of Oregon, Eugene, OR 97403

No abstract provided.

P76 Towards analysis of meiotic telomere functions

Bass, Hank W(1); Danilevskaya, Olga(2); Goltz, Marion(1); Santarella, Rachel A(1) (1) Florida State University, Tallahassee, Florida 32306, USA; (2) Pioneer Hi-Bred Int'l, Johnston Iowa 50131, USA

We seek to understand the roles that telomeres play in meiosis. Telomeres are the specialized ends of linear chromosomes composed of repeated DNA sequences and multiple specific telomeric proteins. Telomeres are involved in cell proliferation and division, and chromosome segregation at meiosis in sexually reproducing organisms. The widely-conserved clustering of telomeres on the nuclear envelope during meiotic prophase produces the bouquet arrangement of chromosomes. The bouquet likely facilitates homologous chromosome recombination and subsequent segregation, or disjunction (Bass et al., 1997 J Cell Biol 137:5-18; Bass et al., 2000 J Cell Sci 113:1033-1042). Improper chromosome segregation at meiosis can produce aneuploid daughter cells. In order to understand the molecular basis of meiotic telomere behavior, we have set out to identify genes that encode telomeric proteins using conserved telomere-binding protein sequence domains to query EST databases. We have identified several candidate cDNAs (M1-M6, P3-P6, IBP1, IBP2) from this computer search approach. These genes will be tested for their expression in meiotic cells, and for their ability to encode telomere binding activity. Results from these studies will contribute to our knowledge of the protein components of maize telomeres.

P77 Development of a high-throughput SNuPE assay to analyse SNPs in the flanking sequence of maize microsatellites

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Analysis has indicated that the flanking regions of microsatellites show considerably more variation than other regions of the maize genome. The observed variation consists of SNPs (Single Nucleotide Polymorphisms) and indels (insertions/deletions). Because of their great abundance and amenability to fully automated genotyping, SNPs and indels are emerging as a new informative generation of markers. We have utilised a high throughput SNuPE (Single Nucleotide Primer Extension) assay to assess 15 polymorphic base variations at 4 microsatellite loci in 24 maize lines, as well as in progeny from a cross between 2 of these lines. Using a MegaBACE automated genotyper, we are able to assay up to 96x13 samples within one hour. This provides valuable high throughput information on genetic relationships among maize genotypes.

P78 Cloning and characterization of a 106 kb region upstream of the B' coding region.
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Paramutation is a natural gene silencing phenomenon whereby interaction between two specific alleles leads to a heritable decrease in gene expression of one of those alleles. At the b1 locus in maize, paramutation occurs when the B-I allele, which confers dark purple pigment, is converted to B', an allele that confers light purple pigment. Paramutation at the b1 locus does not require the B protein and does not involve rearrangements, changes in sequence or methylation state of the paramutated allele within 25 kbp of the coding region (Patterson et al., 1993). In order to identify and characterize sequences at the b1 locus required for paramutation we have used a recombination approach (see abstract by M. Stam et al.). A comparison between recombinant and parental alleles using pulsed field gel electrophoresis showed that the paramutation sequences are located further than 50 kb upstream of the b1 coding region. To be able to map more precisely the recombination sites in different recombinant alleles, a BAC library was made by cloning 100 kb fragment of B' DNA cut with Mlu1. A BAC clone containing 100 kb of sequences upstream of the b1 coding region was isolated and used to generate a restriction map and it was sequenced. The detailed restriction map was used to place 10 contigs derived from shotgun sequencing at 8x coverage. Sub clones were made from that BAC clone and used to isolate single copy probes that have been used to map the recombination break points. Using information from the restriction map of recombinant alleles we have mapped the sequences required for paramutation within 90-103 kb upstream of the b1 coding region. To make a more detailed restriction map of the B', B-I, B-P and b alleles, primers have been designed and used to amplify sequences from different alleles within the 100 kb region. A detailed comparison between the sequences amplified from different alleles is making it possible to narrow down the region where sequences required for paramutation are, and the nature of the sequences suggest hypotheses for the mechanism of paramutation.

P79 Gene Expression Profiling of Two Related Maize Inbred Lines with Contrasting Root Lodging Traits
Bruce, Wesley(1); Desbons, Pierre(1); Crasta, Oswald(2); Folkerts, Otto(2) (1) Pioneer Hi-Bred Intl., Inc., Johnston, Iowa 50131, USA; (2) Curagen Corporation, New Haven, CT 06511, USA

To assist breeding for increased resistance to root lodging in maize, we attempted to identify genes that are associated with root lodging by profiling mRNA expression from two inbreds with contrasting root-related traits. These two inbreds were derived from a common F2 pool, selfed for several generations and showed 75% relatedness based on 106 genetic markers. Under field conditions, the two inbreds exhibited significant differences in root morphology and resistance to root lodging. We collected whole root tissue at two developmental stages from inbred 100 and 101 grown in two years. RNA was isolated from both the V8 and V12 stages. The RNA samples from the 1997-growing season were analyzed by GeneCalling analysis, an open-ended mRNA profiling method. From over 13500 cDNA fragments detected from each of the V8 and V12-stage samples, 229 and 325 cDNA fragments, respectively, showed greater than twofold differences between the two inbred lines. A total of 69 cDNA fragments that showed twofold or greater differences for both inbred lines were observed at both developmental stages. The gene identity and expression differences of several cDNA fragments were determined and confirmed by RNA gel blot analysis. Two genes of five identified were homologous to a cytochrome P450 and the impedance-induced protein, both showing high levels of expression in the roots of lodging resistant lines and low levels in the sensitive lines. These data provide the first clues of genes expressed in roots during the formative stages of development associated with root lodging resistance.

P80 Glume bar phenotypes in a B73 x Mo17 recombinant inbred population reveal the epistatic interaction between pl1 and b1

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The Pioneer SX19 population comprises nearly 1000 recombinant inbred lines derived from B73 x Mo17, of which approximately 380 lines are identical to the IBM population. Although B73, Mo17, and most SX19 lines display green glume bars, many SX19 lines display red glume bars, the intensity of which varies continuously from light pink to dark purple. We treated this trait like a discreet phenotype and scored the SX19 population for the absence or presence of red pigment in the glume bars. We also subjected a number of SX19 lines to additional genetic analysis. From our results we concluded that two unlinked genes condition the red phenotype through an epistatic interaction. Using the IBM population marker data, we determined that pl1-B73 and b1-Mo17 co-segregated with the red glume bar phenotype. We confirmed that these genes are responsible for the red phenotype by genotyping selected IBM lines at pl1 and b1. Our results are consistent with the previously identified functional interaction between pl1 and b1 that conditions anthocyanin pigmentation in the vegetative tissues of the plant, and confirm that B73 and Mo17 carry weak (but not null) alleles of pl1 and b1, respectively. In addition, our results demonstrate the phenotypic effects of an epistatic interaction between weak alleles of two independent genes in a particularly clear manner.

P81 A HIGH EFFICIENY MAIZE "WHISKER" TRANSFORMATION SYSTEM

Bullock, Paul(1); Dias, Dilip(1); Bagnall, Susan(1); Cook, Kathy(1); TeRonde, Sue(1); Ritland, Julie(1); Spielbauer, Dan(1); Abbaraju, Rao (1); Christensen, Jeni(1); Heideman, Nancy (1) (1) Garst Seed Company

The use of silicon carbide „whiskers‰ to genetically engineer maize has now become a very routine and high throughput transformation methodology. Whisker-based transformation is readily capable of delivering either single or multiple genes. Southern analysis suggests that about 47% of the transformation events have single to low copy number insertions. Recent enhancements of the „whisker‰ clone production system are largely based on the use of a more robust and scaled-up „whisker‰/cell collision system. More specifically, a commercial (Red DevilTM) paint shaker is now being used to create collisions within very large batches of cultured maize cells and silicon carbide „whiskers‰. Our current „whisker‰ system scale-up approach allows a single operator to process approximately 3 times the amount of tissue previously addressed, but still during the same 2.5 hour period of time (equivalent to 255 bombardments). Thus, very large numbers of transgenic clones can be produced on a regular basis. This „economies of scale‰ approach to „whisker‰ transformation addresses a number of maize transformation concerns by 1) providing a robust approach that is adaptable to both low and high transformation efficiency cell lines, 2) reducing the cost of labor to produce transgenic clones, 3) maximizing transgenic clone output per unit of time, and 4) advancing breeding time lines through the coordinate development of large populations of unique T-0 transgenic plants that are very similar in maturity. A review of the current status of our maize „whisker‰ transformation system and relevant system scale-up parameters (collision duration, „whisker‰/cell concentrations) will be provided.

P82 The pale aleurone color 1 (pac1) locus, which is required for anthocyanin expression in maize seeds, encodes a homolog of the an11 gene of Petunia and the ttg1 gene of Arabidopsis.
Carey, Charles C(1); Selinger, David S(1); Chandler, Vicki L(1) (1) University of Arizona, Tucson, Arizona 85721, USA; (2) University of Arizona, Tucson, Arizona 85721, USA; (3) University of Arizona, Tucson, Arizona 85721, USA

Mutant alleles at the pac1 locus result in pale aleurone. Previously published work (Selinger and Chandler, 1999 Plant Cell 11, 5-14) demonstrated that pac1 is required for robust expression of the anthocyanin biosynthetic genes and that aleurone color induced by either of the anthocyanin regulators B and R is dependent on pac1 function. Furthermore, experiments in a transient assay system suggested that there was also an effect on the expression of the phlobaphene pathway regulated by P in a pac1 mutant background. Neither b nor c1 RNA levels were affected by the pac1 mutations, suggesting that pac1 functions at the protein level with B and or C1 to activate expression of the biosynthetic genes. We isolated several putative alleles of pac1 from Mutator tagging populations and demonstrated linkage to SSR marker phi087. Using the marker and the mutant phenotype, we observed cosegregation of the mutant allele pac1-ref with a Mu1 element and we used Inverse PCR to recover a substantial portion of the coding region. Another independently isolated allele of pac1 contains a Mu1 insertion in the same genomic fragment, indicating we have isolated the pac1 gene. A number of BAC clones from the B73 BAC library (http://www.genome.clemson.edu/) were identified as containing the pac1 locus. Sequence analysis of the inverse PCR clone demonstrates that the pac1 sequence recovered shares highest similarity with the ttg1 and an11 genes, which play roles in regulation of anthocyanin biosysnthesis in Arabidopsis and Petunia, respectively. Models of pac1 function will be presented.

P83 Mapping Maize Mutants - A Resource of the Missouri Maize Project
Carson, Chris(1); Robertson, Jarrod(1); Bennett, Jennifer(1); Spain, Jake(1); Lewis, Nora(1); Berri, Stefano(3); Hackmann, Pam(1); Melia-Hancock, Susan(1); Neuffer, Gerry(1); Coe, Jr., Ed(1,2) (1) University of Missouri, Columbia, Missouri 65211; (2) USDA-ARS; (3) University of Milan, Italy

Due largely to both the historical cooperativeness among maize geneticists, and the Maize Genetics Cooperation - Stock Center, a large resource of maize mutants is available for gene identification and phenotypic information. A portion of the Missouri Maize Project (MMP) strives to enrich this resource by producing high-throughput map data for maize mutants with SSR markers. We chose to begin with a set of mutants that had been previously mapped to chromosome arms with B-A translocations: mainly those with seedling phenotypes, but also including ones affecting both the seed and adult plant. With 2-point map data, and in many cases multipoint resolution up to the 5cM level, more than 100 of these mutants have been analyzed. The map positions (chromosome, bin, nearest markers) and gene designations for these mutants will be presented. Further, given the massive increase in mapped SSR markers and the information from mutant mapping so far, more informed SSR marker choices help to increase the pace of mutant mapping. In addition, routine identification of previously unplaced mutants is now possible using SSR-PCR to screen pooled samples for band-patterns that reveal linkage (bulk-segregant analysis). We have more than 600 F2 segregating mutant progenies ready for processing, including both placed and unplaced mutants. One chief concern is which mutants should be studied next; thus, all among the maize genetics community are invited to submit their interests and ideas about this issue.

P84 Maize Gene Discovery Project: Microarray Production and Analysis
Galbraith, David W.(1); Chandler, Vicki L.(1); Elumalai, Rangasamy(1); Pierson, Elizabeth(1); Decianne, Dominic(1); Walbot, Virginia(2); Fernandes, John(2); Brendel, Volker(3); Gai, Xiaowu(3) (1) University of Arizona; (2) Stanford University; (3) Iowa State University

See ABSTRACT# W17

MAIZE GENOME WORKSHOP

SATURDAY AFTERNOON

P85 Hierarchical patterns of transgene expression indicate involvement of developmental mechanisms in the regulation of the maize p1 gene
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The maize p1 gene encodes a Myb-homologous regulator of red pigment biosynthesis. To investigate tissue-specific regulation of the p1 gene, maize plants were transformed with constructs combining promoter and cDNA sequences of two alleles that differ in pigmentation patterns: P1-wr (white pericarp/red cob) and P1-rr (red pericarp/red cob). Surprisingly, all promoter cDNA combinations (P::P transgenes) were capable of producing plants with pigmentation patterns similar to a P1-rr allele, with red pigmentation in the five floral organs--pericarp, cob, husk, silk and tassel. However, the P::P transgenic lines also displayed considerably variability in the spatial distribution of pigments among these five organs. Such spatial variation in transgene expression was previously reported for maize plants transformed with a p1 promoter linked to the GUS reporter gene (P::GUS), in which case the patterns fit a developmental hierarchy (Cocciolone et. al., 2000). Application of a statistical test determined that expression patterns of the P::P transgenic plants conform to the same developmental hierarchy observed for P::GUS transgenic plants. Hierarchical expression of both P::GUS and P::P transgenes suggests a possible model for developmental regulation of the endogenous p1 gene. In addition, our results demonstrate that variability in transgene expression, a common occurrence in transgenic plant studies, can be informative if adequately analyzed to uncover underlying patterns of gene expression.

P86 Suppressor of Plant Blotching: A Modifier of the Pl-Blotched allele.
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Pl-Blotched is an epigenetic allele of the anthocyanin regulatory gene Pl that leads to a variegated pattern of pigmentation in both the plant and the kernel. To help understand the epigenetic regulation of Pl-Blotched, we have identified a dominant modifier that alters the pattern of pigmentation normally observed in Pl-Blotched plants. This modifier, designated Suppressor of Plant Blotching (Spb), modifies the amount and distribution of pigment in plant tissues, but has no effect on kernel pigmentation. In the absence of Spb, pigmentation is variegated and locialized mainly in the lower portion of the plant. In the presence of Spb, pigmentation is more uniform and intense throughout the entire plant. RFLP mapping indicates that Spb maps to the short arm of chromosome 9. In addition, RNA blot analysis shows that plants with Spb have increased Pl mRNA and corresponding increases in mRNA for the anthocyanin structural genes. This result is consistent with the more pigmented phenotype of Spb plants and suggests that Spb may regulate the epigenetic state of Pl-Blotched. Because DNA methylation and chromatin structure are know to play a role in expression of Pl-Blotched, assaying these epigenetic indicators in plants with and without Spb should allow us to determine at what level Spb regulates Pl-Blotched.

P87 Modulation of the Rb pathway leads to novel alterations in development
Dilkes, Brian P.(1); Liu, Yan(1); Lowe, Keith(2); Gordon-Kamm, William(2); Woo, Young-Min(1); Larkins, Brian A.(1) (1) University of Arizona, Tucson, Arizona 85721, USA; (2) Pioneer Hi-Bred Intl., Johnston, Iowa 50131, USA

The Retinoblastoma-related (Rb) proteins of plants are hypothesized to be negative regulators of the cell cycle. During seed development, some cells of the endosperm undergo multiple rounds of DNA synthesis without intervening mitoses. This process, referred to as endoreduplication, requires that the initiation of DNA synthesis be uncoupled from mitosis, but other operations such as the maintenance of genome integrity, be preserved. The molecular bases for the unique features of this cell cycle are unknown. Previous work suggested that the Rb proteins regulate maize endosperm endoreduplication. To test this model, the wheat dwarf virus RepA protein, which opposes Rb action, was produced in transgenic maize. While endoreduplication was not changed, gene expression during late endosperm development was altered. Ears from RepA-expressing plants segregated for shrunken opaque kernels, and analysis of RepA-expressing kernels uncovered a deficiency in the production of ADP glucose pyrophosphorylase, all zein storage proteins, and an ABA regulated transcription factor but not a number of other proteins. An interaction was demonstrated between Rb and the chromodomain-containing helicase Pickle (PKL), mutants of which are altered in cell proliferation, GA response, and late seed development. RepA should act as a competitive inhibitor of the Rb-PKL interaction as, like RepA, PKL encodes an LxCxE motif for Rb interaction. Consistent with the phenotypes of RepA transgenic maize endosperms abbrogation of PKL is required for the suppression of the juvenile state in arabidopsis seeds and proper GA signaling. Despite the lack of a cell cycle phenotype in maize endosperm, Rb and RepA act as cell cycle inhibitor and activator, respectively, in other systems. We suggest that Rb is an inhibitor of the cell cycle, but it is not limiting progression of endosperm endoreduplication. However it, or some other target of RepA, is required for correct gene expression during endosperm maturation and or ABA response. Other aspects of the manipulation of the Rb pathway in maize will be described.

P88 SELECTABLE, HERITABLE EPIGENETIC VARIATION AT THE R1 LOCUS

McWhirter, Ken(1); Zinnert, Julie(1); Eggleston, Bill(1) (1) Virginia Commonwealth University, Richmond, VA 23284, USA

Analysis of the stability of the paramutagenic allele R-sc:86, which conditions darkly pigmented seeds and induces weak paramutation, yielded a cluster of seven weakly pigmented (pale) kernels. Repetitive, selective self-pollinations of descendents of one of these pale variants, R-sc:86-17pale, produced semistable sublines with quantitative variation in seed color, ranging from nearly colorless to as darkly pigmented as R-sc:86. R-sc:86-17pale and its sublines were found to no longer be paramutagenic. Southern blot analyses identified no structural differences in or near the three r1 genes in R-sc:86 relative to R-sc:86-17pale and its sublines. However, the r1 genes in R-sc:86-17pale and its sublines were found to have less 5,end methylation and more 3, end methylation than present in R-sc:86. Further, the level of 3, end methylation was found to be roughly inversely correlated with the level of seed pigmentation among R-sc:86-17pale,s sublines. The molecular basis for the pale phenotype was mapped to the 3, end (or more distal) of the proximal r1 gene in R-sc:86-17pale. Current evidence suggests that the quantitative variation in seed pigmentation among the selected sublines of R-sc:86-17pale is due to semistable epiallelism at the r1 locus, rather than to sequence-based changes. This epiallelism appears to be distinct from the epiallelism involved in r1 paramutation, and potentially, could be a basis for quantitative variation at loci throughout the maize genome.

P89 High-throughput Gene Discovery in Maize: Beyond EST

Fu, Yan(1); Liu, Feng(1); Hsia, An-Ping(1); Ashlock, Daniel(1); Schnable, Patrick S(1) (1) Iowa State University

The most common method for high-throughput gene discovery in maize is currently the sequencing of random cDNA clones, i.e., an EST approach. However, genes that are expressed only under unusual conditions or at very low levels will often be missed using this method. Similarly, transposon-based methods of gene discovery will fail to reveal the existence of any genes that are immune to insertion. In contrast, because genes are present at equimolar concentrations in genomic DNA, genomic sequencing potentially provides a means to uncover those genes that will be missed via EST or transposon-based projects. Unfortunately, it is not currently feasible/cost-effective to sequence the entire maize genome. Hence, biochemical approaches need to be developed to filter out the non-coding regions of the genome so that limited sequences resources can be focused on genic DNA. A novel expression vector system was developed to directly rescue open-reading frames (ORFs) from genomic DNA. In a preliminary experiment, 250 genomic fragments cloned into this vector were selected based on a colorimetric screen. Sequence analysis of these clones revealed that 93.6% (234 out of 250) contain an uninterrupted ORF and 55% (129 out of 234) exhibit significant degrees of sequence similarity to entries in protein and EST databases. Many of the remaining clones are thought to contain ORFs that have not yet been discovered via EST sequencing.

P90 Localization, editing and translation of mitochondrial transcripts associated with S-type cytoplasmic male sterility in maize

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The mitochondrial sequences *orf355* and *orf77*, associated with USDA-type cytoplasmic male sterility (cms-S) (Zabala et al. Genetics 147:847), are localized on one transcript. Northern analysis reveals the presence of a 1.6kb transcript, previously implicated in male sterility, in collapsed pollen and not in ear tissue. These data support the gametophytic nature of the cms-S system in maize. Cytidine to uridine (C to U) editing occurs in both *orf355* and *orf77* and a heterogenous mix of edited and unedited transcripts is found in maize pollen. The edit in *orf355* is a silent conversion whereas edits in *orf77* usually result in a change in the coded amino acid. *Orf77* contains regions homologous to *atp9* and to sequence flanking *orf221* (Zabala et al. Genetics 147:847) The *atp9* gene is highly edited (Grosskopf and Mulligan. Curr. Genet. 29:556) and provides and opportunity to analyze out-of-context editing due to its partial presence in *orf77*. A PCR-based screen for editing in *orf77* was developed based upon a C to U edit found in a chimeric region homologous to an editing site in *atp9*. Exploitation of this screen led to discovery of more editing sites including several that introduced premature stop codons. Pollen and ear transcript sedimentation rates are presently being investigated to determine if *orf355* or *orf77* is translated in maize.

P91 OVEREXPRESSION OF PHOSPHORYLATED RAB17 ARRESTS GERMINATION OF TRANSGENIC ARABIDOPSIS SEEDS IN THE PRESENCE OF SALT

Goday, Adela(1); Pagès, Montserrat(1) (1) IBMB. CSIC Barcelona Spain

We have analyzed transgenic Arabidopsis plants expressing the maize Rab17 protein under a constitutive promoter. The protein is found accumulated in all vegetative tissues and shows extensive phosphorylation as occurs with the maize protein, except for the transgenics that bear a construct with a mutated version of consensus motif of the casein kinase 2 where minimal phosphorylation of Rab17 takes place. Under physiological conditions the transgenic plants do not show differences neither in phenotype nor growth in comparison to their untransformed counterparts. However, germination in 100 mM NaCl prevents and arrests the germination of seeds expressing the phosphorylated version of Rab17, whereas the seeds expressing the non-phosphorytable Rab17 protein or the wild type controls are able to complete the germination process. Vegetative tissues of Rab17 overexpressing plants are slighly more tolerant than untransformed controls to high salinity and drought, irrespectively of the degree of phosphorylation of the Rab17 protein. Thus, the function of Rab17 protein during embryogenesis is related to the induction or progression of the dormancy process along the embryonesis, process that occurs in an environment of progressive dehydration.

P92 Paramutation of the p1 locus in maize

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Paramutation is a naturally occurring gene silencing event. Paramutation is defined as an allelic interaction between an inducing (paramutagenic) and sensitive (paramutable) allele that leads to a meiotically heritable reduction in gene expression of the sensitive allele. We have found two alleles of the p1 gene encoding a transcriptional regulator of the phlobaphene pigmentation pathway that participate in paramutation. The paramutable P-rr allele confers dark red pigmentation in pericarp and other floral organs, whereas the paramutagenic P-pr allele, a spontaneous derivative of P-rr, causes reduced pigmentation in the same tissues. Extensive genomic analysis failed to reveal insertions, deletions or restriction site polymorphisms between P-pr and its progenitor P-rr. The reduced pigmentation of P-pr correlates with reduced p1-RNA levels. An enhancer preceding the transcription unit is hypermethylated in P-pr in comparison to the P-rr allele. In addition a DNase hypersensitive site coincides with the hypermethylated sequences. P-pr/P-rr heterozygotes display either a P-pr phenotype or an intermediate level of parental pigmentation. However, the progeny of the F1 x P-ww (a null allele) testcross carrying the P-rr allele predictably retains the pigmentation level of the F1 parent. P-rr,, the P-rr allele after exposure to P-pr, is phenotypically and molecularly indistinguishable from the P-pr allele. Reciprocal crosses reveal no maternal-paternal differences in p1 paramutation. Although P-rr, acquires paramutagenicity and is able to paramutate naïve P-rr alleles, P-rr' is less paramutagenic than P-pr. Expression of P-rr, inversely correlates with cytosine methylation at the enhancer site. Preliminary results show that the P-pr allele when heterozygous with P-rr remains silent. The p1 transcript level in P-pr/P-rr is attributed to the expression of the P-rr allele.

P93 THE CELL DEATH SUPPRESSING FUNCTION OF *lls1*, (*lethal leaf-spot 1*) INVOLVES PROTECTING CHLOROPLAST INTEGRITY.

Gray, John(1); Janick-Buckner, Diane(2); Greenberg, Jean(3); Johal, Guri(4) (1) University of Toledo, Toledo, Ohio 43606, USA; (2) Truman State University, Kirksville, MO 63501; (3) University of Chicago,Chicago, IL 60637; (4) Pioneer Hi-Bred Int. Inc. Johnston, Iowa 50101

TALK ABSTRACT #T18

SESSION 4

ORGANELLES/PLANT DEFENSE

SATURDAY MORNING

P94 Genome-wide Allele-specific Regulation and Heterosis in Maize Hybrids
Guo, Mei(1); Rupe, Mary(1); Smith, Howie(1); Yang, Sean(1); Bowen, Ben(2); Crasta, Oswald(3) (1) Pioneer Hi-Bred International, Inc. 7250 NW 62nd Ave, Johnston, IA 50131-0552; (2) Lynx Therapeutics Inc., 25861 Industrial Boulevard, Hayward, CA 94545; (3) CuraGen Corporation, New Haven, CT

Heterosis can be described as the increased vigor of hybrid progeny compared to their inbred parents. The molecular mechanisms underlying this phenomenon remain a mystery. Recently, genome-wide RNA profiling of pre-pollinated immature ear tissue of maize hybrids and their inbred parents via CuraGen,s GeneCalling technology has provided some insights. With the aid of this open-ended mRNA profiling technology, gene expression and genetic mechanisms associated with heterosis can begin to be examined. In addition, the capability of this technology to detect not only gene expression level differences, but also allele sequence polymorphisms enables one to quantitate allele-specific expression. For the majority of the genes profiled, the level of expression in the F1 fell within the range of the two parents, with a bias towards the paternal level in nearly all (30) hybrids examined. This deviation from the mid-parental expression is interesting because it is negatively correlated with heterosis. The „paternal biased expression‰ increased in low yielding hybrids and hybrids grown under stressed environments, while decreasing in heterotic hybrids and hybrids grown under high yielding environments. Deviations from the mid-parental expression indicated by CuraGen profiling for selected genes were confirmed with RT-PCR in which silencing or partial silencing of one parental allele was found. This data indicates the connection of allele-specific silencing with heterosis and environmental stresses. Allele-specific silencing suggests epigenetic mechanisms might be involved in such global gene regulation.

P95 mRNA Profiling of Maize Aneuploids Using cDNA Microarray
Zhao, Suling(1); Birchler, Jim(2); Guo, Mei(1); Yang, Sean (1); Dieter, Jo(1) (1) Pioneer Hi-Bred Int. Inc., 7250 NW 62nd Ave, Johnston, IA 50131-0552, USA; (2) University of Missouri, Columbia, MO 65211, USA

Addition or subtraction of a chromosome relative to the normal genomic complement often causes a reduction in plant vigor. Using Northern hybridization, we have previously studied the effect of the dosage series of 14 chromosomal segments on six genes (Adh1, Adh2, Glb1, Sh1, Sus1, 22 KD-Zein) expressed in the endosperm. Results from this study support the hypothesis that the aneuploid syndrome was the consequence of an altered dosage-sensitive regulatory system. Recently developed high-throughput technologies allow one to examine the expression of a large number of genes in aneuploids simultaneously. We tested the feasibility of using microarrays for this purpose by profiling the dosage series of chromosome arms 7L and 10L. Hypoploidy of 7L and 10L is associated with reduced kernel size. Examining gene expression of the endosperm tissue of such aneuploids and their euploid counterparts will help us identify genes affecting kernel size. A microarray with 1,400 ESTs was used for this experiment. Most of these ESTs are regulatory genes. Approximately 7% of these genes exhibited either a direct effect (expression increased with increased chromosomal dosage) or an inverse effect (expression decreased with increased chromosomal dosage). There were more genes that exhibited a direct effect than an inverse effect in endosperm, which is consistent with the previous Northern data. The function and role of these genes in endosperm development remain to be determined. These preliminary results indicate that microarray analysis can be a useful tool for screening and studying gene expression differences in a dosage series.

P96 Isolation of maize genes with an imprinted pattern of expression.
Gutierrez-Marcos, Jose F.(1); O'Shea, Suzanne(1); Vanderpump, Sarah(2); Greenland, Andy (2); Dickinson, Hugh(1) (1) Dept. Plant Sciences, Oxford University, Oxford, OX1 3RB, UK.; (2) Syngenta, Jealotts Hill Research Station, Bracknell, Berks, RG12 6EY.

Talk #25 session 5 the Gene Sunday monrning

Endosperm is the product of a double fertilization process that occurs in most angiosperms. The egg and one of two sperm cells fuse to produce the embryo, while the two polar nuclei of the megagametophyte fuse with the other sperm to generate the triploid primary endosperm nucleus. Development of the endosperm in most angiosperms is required for the viability of the embryo and it has been demonstrated that the genomic ratio 2:1 (2 maternal:1 paternal) can be crucial for the successful development of this tissue. Any divergence from this ratio normally results in abortion of the endosperm. The molecular and cellular consequences for parental genomic interactions are thus highly important. A molecular mechanism must exist to sense the balance between the contribution of both parental genes, in that when an incorrect balance is detected, development is arrested. There is accumulating evidence that this molecular mechanism involves a system of gametic imprinting. Gametic imprinting is a unique form of epigenetic inheritance by which expression of certain genes, from generation to generation, is governed by their parental origin. Perhaps the most striking feature of imprinted genes is that active and inactive parental alleles coexist within individual cells. In plants, current evidence suggest that the embryo is less susceptible to the effects of gametic imprinting, than the endosperm. To date, only four different genes have been found to be imprinted in the plant endosperm, and in every case, maternally-inherited alleles are undermethylated and highly expressed. In order to explore the role that gametic imprinting plays in endosperm development, and to reveal the molecular mechanisms involved, we have identified and characterised two maize gene classes that present opposite parent of origin patterns of expression in the maize endosperm.

P97 Genetic components required for paramutation at the pl1 locus
Hollick, Jay B.(1) (1) University of California, Berkeley

Genetic analysis of paramutation at the purple plant 1 (pl1) locus, a regulator of the anthocyanin biosynthetic pathway, is ongoing to gain insights into general chromosome processes used in gene control. Paramutation describes a directed, and heritable, alteration in gene regulation that appears to occur without DNA sequence changes. One particular pl1 allele, Pl-Rhoades (Pl-Rh), is unstable and can spontaneously change to a weaker expression state called Pl,. The Pl, state is both mitotically and meiotically stable and invariably causes paramutation of other Pl-Rh alleles present in the same nucleus. Stable maintenance of the silenced Pl, state requires undefined trans-interactions with a second Pl-Rh or Pl, allele (Hollick and Chandler, Genetics 150: 891). Genetic screens have identified at least eight distinct trans-acting factors whose normal functions are also necessary to maintain the silenced Pl' state: mop1 (mediator of paramutation 1; Dorweiler et al., The Plant Cell 12: 2101), rmr1 and rmr2 (required to maintain repression; Hollick and Chandler, Genetics 157: 369), rmr6, rmr7, rmr8, rmr9, and rmr11. At variable frequencies, Pl, can heritably revert to the fully expressed Pl-Rh state in homozygous rmr1, rmr2, rmr6, rmr9 and rmr11 mutant sporophytes but not in homozygous rmr7 or rmr8 mutants. The maize complement of rmr factors and the effects of rmr mutations on Pl' silencing and normal plant development will be summarized. The diverse effects of rmr functions are consistent with their proposed role in general regulation of genome homeostasis and function.

P98 A tranfer cell myb-related gene activates the expression of previously described transfer cell specific genes.

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In the course of a search for genes expressed at the basal endosperm transfer cell layer of maize we have isolated a gene, ZmMRP-1, encoding a myb-like DNA binding domain. ZmMRP-1 is a single copy gene that maps in the chromosome 8 of maize. Northern, RT-PCR and „in situ‰ hybridisation analyses demonstrated that the gene is exclusively expressed at the transfer cells of the maize endosperm. ZmMRP-1 expression is detected in the cytoplasmatic domains surrounding nuclei at the base of the endosperm syncitium as early as 2 days after pollination. Localisation of a translational fusion to GFP supports that ZmMRP-1 is a nuclear protein. Co-transformation experiments in tobacco protoplasts and particle bombardment of transgenic plants demonstrated that the ZmMRP-1 protein efficiently transactivates transfer cell specific promoters.

P99 "Digital Northern" analysis of publicly available cDNA libraries

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Determining ESTs frequencies in a standard cDNA library is a way to evaluate the expression level of the corresponding transcripts. If this scoring is conducted in several libraries it becomes possible to compute digital expression profiles. By considering a large number of EST, multivariate data analysis methods can reveal a limited set of expression patterns. In this work, we used hierarchical clustering, PCA and SOM to analyse numerous public maize libraries (data were downloaded from ZmDB). We first worked on how to choose ESTs to be included in our data set. Then we focused on a cluster obtained from hierarchical clustering and observed how its EST members were classified by the two other methods. Detailed bio-informatics analyses were performed on each member. As this cluster was made of well characterized and unknown ESTs we tried to evaluate to what extent digital northern can help assigning putative function to these unknown ESTs.

100 High efficiency Agrobacterium-mediated T-DNA transfer into maize immature embryos.
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Development of efficient, user-friendly maize transformation methods is critical for expanding high-throughput functional genomics analysis in maize. Gene transfer via Agrobacterium is a preferred method for transgenic plant production and genomic research because inserted transgene copy numbers are low, resulting in more stable transgene expression. Use of supervirulent Agrobacterium strains and superbinary vectors (vectors containing extra copies of the vir genes) has recently made it possible to transform maize via Agrobacterium. The objective of our research was to optimize several chemical and physical parameters of cocultivation to achieve efficient Agrobacterium-mediated TDNA transfer and integration could be achieved in maize without the use of proprietary superbinary vectors. Among the various factors tested, temperature and culture media composition greatly affected the T-DNA transfer as evidenced by transient expression of GFP and GUS proteins (int-Gus). A range of co-culture temperatures (10, 15 18, 20, 22, 25 and 27 oC) was tested using immature embryos from three maize genotypes. The highest efficiency of T-DNA transfer occurred at 20C - 22 oC. As high as 90-95% of explants showed GUS expression and 75% of the total area stained blue at 20 oC. No GUS expression was reported at 10 and 15 oC, and only low frequency expression was observed at 18 oC. Increases in co-culture temperature above 22 oC inhibited Agrobacterium infection and T-DNA transfer. Another factor exerting a strong effect on efficiency of Agrobacterium-mediated T-DNA transfer in maize was the concentration and type of basal salts used in the coculture media. Immature embryos from four maize genotypes were cocultured on media varying in basal salt type (N6 or MS) and concentration (full or half strength). Transient expression results indicated a genotype by salt type/concentration interaction. Thus, high frequency T-DNA delivery depended on specific media/genotype combinations.

P101 Development of Transgenic Maize with Altered Linoleic/Oleic Acid Content
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We are testing several gene constructs in maize designed for inhibition of conversion of oleic to linoleic acid to study gene expression of fatty acid desaturase2 (fad2) isoforms and to enhance the nutritional composition and oxidative stability of corn oil. Microprojectile bombardment of HiII (type 2) callus with either sense or anti-sense fad2 constructs driven by the ubiquitin promoter (constitutive), yielded transformed callus that regenerated into mature plants. Fatty acid analysis, by gas chromatography, of 20 embryos from seed of regenerated (R0) plants transformed with a ubiquitin/ fad2 anti-sense clone, generally indicated percentages of oleic acid to linoleic acid similar to wild type control embryos, 22% oleic acid and 62% linoleic acid. However one embryo was significantly altered with 72% oleic acid and 5% linoleic acid. Two other samples showed less profound changes in their fatty acid profiles with 26%/60% and 28%/58% oleic/linoleic. Analysis of leaf samples for altered 18:1/18:2 profiles showed smaller changes possibly due to the large amount of 18:3 (linolenic acid), ~72%, compared to approximately 1% in the embryo. A 1400 bp. fragment 5, to a maize fad2 gene was isolated by inverse PCR and cloned into a transformation cassette carrying the GUS gene. Microprojectile bombardment of 22-day-old (days after pollination) H99 maize embryos with the 5, 1400 b.p. fragment-GUS construct showed positive histochemical staining for GUS gene activity, indicating promoter activity of the 5, fragment in the immature maize embryo. The 5' 1400 b.p. fad2 fragment-GUS was also used to transform H99 embryogenic callus. Several plants have passed through selection and have been regenerated. The impact of reduced fad2 activity in transgenic maize upon kernel oil composition and photosynthesis is being studied.

P102 Identifying Cross-Taxa Pcr Generated Markers For A Tripsacum Dactyloides Mapping Population

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Fertile cross compatibility between Tripsacum dactyloides and Zea species offers unique breeding opportunities for bi-directional transfer of novel genes. Tripsacum genetic contributions to maize can lead to important advances in improved grain nutritional quality, new resources for resistance to environmental stresses, and apomictic reproduction. Similarly, maize derived genes are expected to enhance such characters as seed set and germination in Tripsacum. Comparative genomics involving T. dactyloides and marker assisted breeding programs for agronomic and scientifically important traits requires the construction of a genetic molecular map with markers well distributed over the 18 Tripsacum linkage groups. This study tested the utility of maize and sorghum derived DNA primers to detect PCR generated markers for a diploid T. dactyloides F2 mapping population. Amplification by 79 maize SSR primers was 63% with similar results obtained using primers designed to 26 maize ESTs. Surprisingly, many Tripsacum amplicons were significantly higher in molecular weight when compared to their maize counterparts. The 18 tested sorghum SSR primers resulted in approximately 55% amplification success, but required modified reaction conditions. SSLP type markers useful for mapping were infrequent, a consistency with the polymorphism reductions often observed when attempting cross-taxa PCR based methodologies. Heterozygosity and allelic transmission from the parental genomes to the F1 parent increased the difficulty to discover PCR primers which produced informative Tripsacum SSLPs. Consequently, two alternative strategies were investigated to determine if useful polymorphism identification could be significantly enhanced. The results on cleaved amplified polymorphic sequence (CAPS) detection by 4 different endonucleases, and preliminary sequence analysis of parental amplicons will be presented.

P103 Isolation of methyl-CpG-binding domain genes from maize.

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Mammalian methyl-CpG-binding domain (MBD) proteins are involved in nucleating silencing complexes and repairing spontaneous deamination at methylated CpG dinucleotides. The objective of this study was to characterize the MBD proteins present in maize. The MBD from the mammalian protein MeCP2 (amino acids 93-149) was used to search the Arabidopsis genome and the maize EST collection for sequences containing a putative methyl-CpG-binding domain. A total of eleven Arabidopsis proteins containing an MBD were found. These eleven proteins fall into six classes based on sequence within the putative MBD and outside this domain. Searches of the maize EST database revealed four contigs with similarity to Arabidopsis MBD proteins. We obtained full-length sequence of the maize genes MBD101, MBD105, MBD106, and MBD108. These four maize genes represent three of the six classes of MBD proteins found in Arabidopsis. The methyl-CpG-binding domain found in mammalian proteins contains four beta sheets, two loops and an alpha helix. The putative methyl-CpG-binding domains from all plant MBD proteins contain similarity to these structural regions. There is no similarity detected between plant and animal MBD proteins outside the MBD domain itself. Phylogenetic analysis of plant and animal MBD sequences indicates that the plant MBD sequences are more closely related to one another than they are to mammalian sequences. This indicates that molecular components that interpret DNA methylation patterns may be quite different in plants and animals.

P104 Analysis of zein interactions and their role in protein body formation
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Zeins are the most abundant proteins in maize endosperm, accounting for 70% of the total protein. There are four structurally distinct types of zeins: the sulfur-poor alpha-zeins and the sulfur-rich beta-, gamma- and delta-zeins. These proteins form 1~2µm accretions, or protein bodies, within the lumen of the rough endoplasmic reticulum (ER). The mechanisms that bring zeins together, cause protein bodies to form as discrete entities in particular ER locations and grow to a uniform size are unknown. However, our research supports the hypothesis that factors influencing the number of protein bodies and the organization of zeins within them contribute to the formation of a normal, vitreous endosperm phenotype. To investigate the nature of zein interactions within the ER, we expressed a gene encoding each type of zein protein individually and in combination in yeast cells. The zeins were produced as native proteins, or GFP fusions, using highly expressed yeast promoters. The yeast two-hybrid system was also used to test protein-protein interactions between pairs of zeins. These experiments show previously uncharacterized interactions within each zein class. We identified the smallest region of a 22-kD alpha-zein that interacts with other zein proteins.

P105 Analysis of the anthocyanin structural gene - Whp in Zea mays
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The Whp gene and the C2 gene encode chalcone synthase (CHS), a key enzyme in anthocyanin biosynthesis. The full size cDNA of the Whp gene has been cloned from an in,c,Whp background. Sequence comparison revealed several differences of the cDNA-and amino-acid sequence to the C2 gene and to the genomic Whp sequence known so far. Expression analysis of Whp in various genetic backgrounds revealed enhanced expression of the gene in the Line C1-S. The quantification of the Whp gene expression in different tissues was made by RT PCR. The results showed, that in kernels from C1- S plants Whp gene transcription is enhanced as compared the W22 inbred line Line C. In order to find out, whether expression differences could result from promotor differences, the Whp promoters of C1-S and LC were isolated and analyzed. Several sequence alterations were found comparing the Whp promotor- and 5`-UTR sequences of LC and C1-S. For further promotor analysis luciferase reporter-gene constructs were analyzed by transient expression in maize scutellum. These data show that Whp expression is influenced by the transcription factors C1, R and In (In-D.

P106 A new molecular marker technique: Using Resistant gene analogues (RGA) and Miniature-inverted repeat elements (MITEs).
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A new molecular marker technique was developed using Resistant gene analogues (RGA) and Miniature-inverted repeat elements (MITEs). Oligonucleotide primers based on the original sequences of the two MITE elements, Heartbreaker (Hbr) and Hb2, were used together with primers based on the conserved motifs of different gene protein sequences in a modified Transposable-display (TD) approach to amplify fragments in the maize genome. Applying this modified Transposable-display method, thirty-two primer combinations were used to amplify a total of 380 scorable fragments. Two hundred and eleven (211) fragments were polymorphic. Of these, 197 could be mapped on one of the ten maize chromosomes, using 40 recombinant inbred individuals derived from a cross between Tx303 x C0159. Mapping results showed that these fragments occur in clusters spread across the ten chromosomes. Chromosome 2 had the most mapped fragments (39), in comparison to chromosome 9 with only nine fragments. Ninety-two of these fragments mapped in the proximity of known disease- or insect resistance genes or Quantitative trait loci (QTLs). Further analysis using well-defined models for certain disease resistance genes strongly suggested that some of these novel markers might be referred to as candidate genes.

P107 Optimization of Mu-TAIL PCR for analysis of Mu flanking DNA from high-copy lines
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Because of their capacity for generating high mutation frequencies and strong preference for transposing into genes, high-copy DNA transposons such as Robertson's Mutator (Mu) provide powerful tools for functional genomics. We have focused on developing efficient strategies for molecular analysis of high copy transposons in the maize genome. We have shown previously that the Thermal Assymmetric Interlaced (TAIL) PCR protocol allows efficient amplification of Mu flanking DNA from high-copy maize lines. Because the existing protocol was originally optimized for the Arabidopsis genome, there is potential for improving application of TAIL to amplification of maize DNA. In addition, key genomics applications of Mu-TAIL require an accurate estimate for the fraction of the total population of Mu insertions within the genome that are represented in the Mu-TAIL amplicons. To address these issues we have developed an assay for the representation of Mu-TAIL amplicons based on analysis of ten diverse Mu tagged genes. Using this assay and a rational approach to primer design, we have developed new arbitrary primers that significantly broaden the representation of Mu-TAIL. Our results indicate that Mu-TAIL primers can be optimized to achieve an arbitrarily high average representation of Mu insertions in a sample of genomic DNA.

P108 USE OF [F]dNTP METHOD TO EXPLORE THE PROBLEM SOURCE FOR LOW SIGNAL INTENSITY ASSOCIATED WITH HEX-LABELED MAIZE SSR MARKERS

Lee, Warren(1); Yue, Yong(1) (1) Dow AgroSciences LLC, Indianapolis, Indiana 46268, USA

USE OF [F]dNTP METHOD TO EXPLORE THE PROBLEM SOURCE FOR LOW SIGNAL INTENSITY ASSOCIATED WITH HEX-LABELED MAIZE SSR MARKERS Warren Lee and Yong Yue. Department of Trait Development, Dow AgroSciences LLC, 9330 Zionsville Road, Indianapolis, IN 46268. Fluorescently labeled primers are routinely used for the detection of PCR fragments using ABI377. In our observation, HEX-labeled primers generally yield lower signal intensities compared to FAM or NED-labeled primers, which may impact the data quality and result in data loss. Lower primer specificity and efficiency in a PCR reaction is another cause of sub-optimal data quality. To test whether the reduced signal intensity associated with HEX-labeled products was due to the label itself or due to sub-optimal PCR primers, we have conducted a study using 130 maize SSR primer pairs that yielded poor results when Hex-labeled primers were used. PCR products for all 130 markers were amplified using [F]dNTPs and unlabeled primers from four different genotypes. Labeling PCR products with [F]dNTPs in PCR is an alternative method for the detection of the expected peaks that could have otherwise been missed with HEX-labeled primers. The results were compared to their HEX-labeled counterparts. While the products of 45 (35%) primer pairs recorded enhanced peak heights, 15 (11%) of them displayed similar performance with both [F]dNTPs as well as HEX. The remaining 70 (54%) did not work with either of the approaches indicating sub-optimal primer characteristics and the need to redesign primers. The forty five markers that were successful with [F]dNTPs also performed well when FAM, NED or VIC-labeled primers were used for PCR, further emphasizing that their previous failure was due to HEX-related properties.

P109 Identification of genes induced during early kernel development in Zea mays (L.)

Lorbiecke, René(1); Kukula, Janine(1); Paul, Carina(1); Wienand, Udo(1) (1) Institut für Allgemeine Botanik, AMP I, Universität Hamburg, Germany

The identification of seed specific genes from maize endosperm is currently of growing interest to provide more efficient approaches for plant improvement. To get more insight into the regulation of endosperm and kernel development we performed a PCR-based cDNA subtractive hybridization in order to identify genes induced early during maize kernel development. cDNAs for driver and target populations were synthesized using mRNAs from kernels 0 and 8 days after pollination. The enriched cDNA fragments were cloned and further screened for differentially regulated genes by dot blot hybridization using driver and target cDNAs as probes. Based on this screening we estimated that about 30-40% of the subtraction-enriched cDNAs represent differentially regulated genes. Sequencing revealed that all cDNA fragments so far cloned are different in sequence. Northern analysis confirmed an induced and transient expression pattern of the genes analyzed, showing the efficiency of the subtraction and screening procedure. In addition, most of the isolated genes showed strongest expression in developing kernels and weak or no expression in other tissues analyzed i.e. tassels, silks, leaves or young plants. Database search lead to the identification of new genes involved in lipid metabolism and pathogen response as well as genes already known to be endosperm specific, i.e. BETL2.

P110 Molecular Analysis of a Lem(Lethal Embryo) mutant in maize
Ma, Zhengrong(1); Dooner, Hugo(1) (1) Rutgers University, Piscataway, NJ 08854, USA

A recessive lem mutant was isolated form a collection of maize plants carrying transposon Ac. Homozygous mutant embryos abort earlier than 10 DAP, but kernels develop relatively normal endosperms. Genetic analysis strongly suggests that it is an Ac -tagged mutant. Southern blot using Ac as a probe detected a unique band that cosegregates with the mutant phenotype. DNA flanking Ac was cloned and mapped to chromosome 1L with recombination inbred lines. A full length cDNA was obtained by cDNA library screening and 5, RACE. A size-fractioned genomic library was constructed and screened to get the wildtype allele. This gene has very high homology to the rice chloroplast 30S ribosomal protein S9 gene. We are currently investigating how a nuclear-encoded chloroplast gene could cause the abortion of the embryo.

P111 Isolation and characterization of sperm-expressed genes in *Zea mays*
Engel, Michele L.(1); Chaboud, Annie(2,3); Dumas, Christian (2); McCormick, Sheila (1) (1) Plant Gene Expression Center, USDA/ARS-UC-Berkeley, 800 Buchanan St., Albany, California 94710, USA; (2) Laboratory of Plant Reproduction and Development, UMR 5667, Ecole Normale Supérieure de Lyon, CNRS-INRA-ENS, Lyon-UCB Lyon I, 69364 Lyon Cedex 07, France; (3) Flow Cytometry Facility, Ecole Normale Supérieure de Lyon, Lyon, Cedex 07, France

see TALK ABSTRACT # T24

SESSION 5

THE GENE

SUNDAY MORNING

P112 Serial Analysis of Gene Expression assay of developing kernels from the Illinois Long Term Selection Oil Strains.

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Researchers at University of Illinois have been selecting for high and low oil since 1896 from the open pollinated variety Burr's White. Illinois High Oil (IHO) and Illinois Low Oil (ILO) differ quantitatively in the oil composition, and in the embryo size, phenotype and other developmental traits. Several QTL have been shown to influence the oil concentration. The Serial Analysis of Gene Expression (SAGE) assay is being used to study the expression profiles of the Illinois strains that may show quantitative transcript differences between the high and low oil strains which have been divergently selected for 100 years. SAGE strategy is also being used to study the expression profiles of IHO derivatives, Reverse High Oil and Switchback High Oil and also the ILO derivative, Reverse Low Oil. SAGE technique is being used to screen libraries constructed from embryos harvested at 14, 21, 28 and 35 days after pollination from the above mentioned strains. Thus a SAGE survey comparing the transcript levels from multiple strains along with the developmental stages could possibly identify candidate genes involved in the deposition of higher oil in the embryos. In this on going research, several hundred SAGE tags have been obtained from IHO. SAGE library construction and sequencing is in progress for the other strains. Our target is to obtain approximately 15,000 SAGE tags or more from each individual SAGE library which could provide a good metric for identifying and measuring transcript level differences between the strains.

P113 High Efficiency Transgene Segregation in Co-transformed Maize Plants using an Agrobacterium tumefaciens Two T-DNA Binary System

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For certain regulatory issues and research purposes it would be desirable to have the ability to segregate transgenes in co-transformed maize. A highly efficient system to segregate transgenes in maize co-transformed using an Agrobacterium tumefaciens two T-DNA binary system is reported. Three vector treatments were compared in this study; 1) the selectable marker gene bar and GUS reporter gene are on two separate T-DNA,s contained on a single binary vector 2) a strain mixture where bar and GUS are contained on single T-DNA,s in two separate strains 3) a single T-DNA binary vector containing both bar and GUS. Transgenic callus was generated from 52-59% of infected immature embryos depending on treatment. GUS was co-expressed in T0 plants generated from this callus at a rate of 87% for the two T-DNA vector, 10% for the mixed strain treatment and 99% for the single T-DNA vector containing both bar and GUS. A total of 87 co-expressing T0 events from the two T-DNA binary vector treatment were evaluated for segregation of bar and GUS at the T1 generation. From these T0 events, 71.4% exhibited segregation of bar and GUS at the T1 generation. A total of 64.4% of the two T-DNA vector co-expressing events produced selectable marker-free T1 progeny. A high frequency of segregation was also observed using the mixed strain approach but a low co-expression frequency at the T0 generation makes this method less efficient. A high efficiency system to segregate transgenes in co-transformed maize plants has now been demonstrated.

P114 High throughput genotyping of single nucleotide polymorphism by bar coded oligonucleotide ligation assay **Peng, Jiqing(1); Bongard-Pierce, Deverie(2); Davidow, Lance(2); Golt, Caroline(1); Lemieux, Bertrand(1)** (1) Department of Pland and Soil Sciences, University of Delaware, Newark, Delaware 19717-1303, USA; (2) Deaprtment of Molecular Biology, Massachusetts General Hospital, Boston, MA 02114, USA.

Single nucleotide polymorphisms (SNPs) are the most frequent type of sequence variation found in eukaryotic organisms. We have developed a novel approach to score the inheritance of SNPs that uses oligonucleotide ligation assays (OLA) adapted to DNA chip technology. OLA exploits the fact that DNA ligase will join two oligonucleotides that are properly aligned by a "guide" DNA but will fail to do so if the 3' end of one of the oligonucleotides is mispaired with the guide (Landegren et al. 1988). Indeed, this mispairing causes a misalignment of the 3'end of the first oligonucleotide (a.k.a. allele specific oligonucleotide or ASO) relative to the 5'end of the second oligonucleotide (a.k.a. locus specific oligonucleotide or LSO). If this guide DNA contains a SNP, each allele of the SNP will be detected by the creation of ligation products containing different ASO and the LSO to distinguish which ASO has been ligated to a LSO with a fluorescent reporter group. The assay we have developed is called the bar coded oligonucleotide ligation assay (BOLA) because it use a set of 4,500 "bar code" hybridization tags (Shoemaker et al. 1996) to target the products of the OLA reactions to specific addresses on an oligonucleotide microarray containing bar code complementary sequences. The ASO used for BOLA each have a distinctive bar code sequence. A set of SNPs that were identified by mining publicly available sequence traces (Gai et al. 2000; http://www.zmdb.iastate.edu/) with SNP finder (Altshuler et al. 2000). Script was written to extract 40 nucleotides on each side of these cSNPs in order to design primers to amplify each SNP. The SNPs were amplified by multiplex PCR and the resulting products used as guide DNA for BOLA. The BOLA products are then hybridized to the microarray and the hybridization signal is detected by laser confocal scanning. This system permits the detection of two alleles using high throughput and robust format. It can be broadly applied to genome-wide linkage mapping in any organism.

P115 Molecular Analyses of a Maize Centromere
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Centromeres are cytologically defined as the primary constriction of a chromosome. Functional centromeres are required for proper chromosome segregation during cell division. In plants, several sequences have been identified as centromeric but no sequences have been shown to be critical for function. We are interested in the maize B chromosome centromere. Previously, it has been shown that the B centromere contains a specific sequence repeated many times in a degenerate array. The B centromere is an ideal model for studying centromere function because the B chromosome is dispensable. Therefore, reducing the transmission of the B chromosome by disturbing the centromere does not reduce the viability of the plant. In fact, a univalent B chromosome undergoes a natural centromere reduction through misdivision. Misdivision occurs when the univalent chromosome is pulled by spindle fibers from both poles causing the centromere to break. Successive misdivisions have reduced the size of the B centromere from the original 9 Mb to 90 Kb. Because the misdivided chromosomes are still transmitted, the regions of the centromere responsible for function must be present. We study the structure of misdivision derivative centromeres in order to determine what regions are critical for function. In this report, the molecular structure of the misdivision derivative centromeres has been further characterized and several new misdivision derivatives have been identified. We have also begun cloning and sequencing regions of the derivative centromeres in order to identify other sequences present and to determine their organization. Finally, we have taken two approaches to developing an assay that will allow us to test the function of centromeric sequences in plants. In the first approach, we transformed a modified BAC vector that contains centromere DNA and maize selectable markers. In the second approach, we co-transformed all the necessary components believed to be required for chromosome function in order to determine if a chromosome could be assembled *de novo*. In either case, the existence of an additional chromosome or the formation of dicentric chromosomes will indicate that the transformed centromeric sequences are functional.

P116 The Etched 1 gene product of Zea mays contains a zinc ribbon-like domain and is homologous to the eucaryotic transcription elongation factor TFIIS

da Costa e Silva, Oswaldo(1,4); Garg, Preeti(1); Waßmann, Martina(1); Lorbiecke, René(1); Lauert, Patricia(1); Peters, Ulrike(1); Scanlon, Mike (2); Hsia, An-Ping(3); Schnable, Patrick S.(3); Wienand, Udo(1) (1) Institut für Allgemeine Botanik, AMP I, Universität Hamburg, Germany; (2) University Georgia, 3609 Plant Sciences, Athens GA 30602, USA; (3) Iowa State University, Ames, IA 50011, USA; (4) BASF Plant Science LLC, 26 Davis Dr, Research Triangle Park NC 27709, USA (present address)

see TALK ABSTRACT #T11

SESSION 3

THE SEED

SATURDAY MORNING

P117 Sequence diversity at the yellow endosperm (y1) locus of maize and the origins of yellow phenotype of cultivated corn

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Phytoene synthase, an enzyme of carotenoid biosynthesis pathway, is responsible for the yellow endosperm phenotype of maize (BUCKNER et al. 1998). Most of the cultivated maize grown in the United States and Europe have yellow endosperm color, which is determined by the dominant phytoene synthase / y1 locus. A small amount of white (recessive y1 allele) is grown for food uses. White corn is preferred in some countries. Conversion to yellow endosperm varieties occurred relatively recently with the realization of the nutritional advantages of carotenoid content of yellow corn, especially in animal feed. The y1 gene was transposon-tagged (BUCKNER et al. 1990), cloned (BUCKNER et al. 1998), and the diversity of the (CAA)n microsatellite in maize and teosinte was investigated (PHELPS et al. 1996). Here we analyze genetic diversity at the phytoene synthase locus in white and yellow agronomic lines of corn by DNA sequencing of multiple white and yellow alleles of y1. We show that DNA sequence diversity of white corn is significantly higher than that of yellow corn at the y1 locus. All yellow lines we analyzed contain an Ins2 insertion in the promoter of the y1gene, while none of the white lines have the Ins2 element at that locus. A considerable linkage disequilibrium exists within the 5 kb region of y1 in the germplasm we analyzed. These observations are consistent with the hypothesis that white germplasm is ancestral relative to yellow and that a single Ins2 insertion event may be responsible for the majority or all of cultivated corn yellow phenotypes. The insertion itself may act by providing a transcription factor binding site that activates endosperm expression of phytoene synthase, in addition to pre-existing seedling and leaf expression. An alternative hypothesis would involve inactivation of the binding site for a negative regulator of endosperm expression by the Ins2 insertion. Further studies of a wider range of germplasm, including exotic cultivated maize, may identify other sources of yellow phenotype. We are also examining the extent of linkage disequilibrium at the y1 locus, taking advantage of the availability of BAC clones in the region (E. Ananiev, unpublished observations)

P118 NUCLEOTIDE DIVERSITY AND PHENOTYPIC ASSOCIATIONS FOR TWO TRANSCRIPTIONAL ACTIVATING FACTORS OF ZEIN PROTEINS

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As the maize kernel develops, approximately 50% of the protein that accumulates in the endosperm is zein, also known as prolamins, alcohol soluble proteins that are encoded by upwards of 75 genes. Opaque 2 (o2) and prolamin binding box factor (pbf) are two examples of many possible transcriptional factors responsible for coordinately regulating protein accumulation during the development of the maize kernel. Both proteins are only expressed in the developing endosperm. While the o2 locus encodes a basic leucine zipper (bZip) that binds as a dimer to the promoter of the 15- and 22 kDa zein genes, the pbf locus encodes a zinc-finger protein that binds to the P-box, present in the promoter site of all zein class genes. Each gene was sequenced from 32 diverse maize inbred and 10 teosinte lines. Linkage disequilibrium was low in both genes. Where present, significant LD ($P<0.001$) between polymorphisms decayed within 1kb of sequence. Nucleotide diversity was calculated for each gene, o2 showed high diversity across the entire gene in all lines. Pbf showed very low diversity in all maize lines, while, diversity was 7 fold higher in Z. m. ssp. parviglumis, suggesting that pbf may be a domestication gene. Phylogenetic analysis demonstrated that inbreds were closely related to one another while teosinte lines had a wide range of alleles. Tajima's test statistic and HKA test for selection were significant for pbf suggesting selection during the domestication of maize. Association tests were carried out to identify putative functional polymorphisms having an effect on phenotypic variation in kernel traits.

P119 Maize / Sporisorium reilianum zeae interaction

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Sporisorium reilianum zeae is the causal agent of maize head smut. Although this fungus is not the most important pathogen of maize, this basidiomycetous fungus is endemic in many countries all over the world. We first investigated the peculiar biology of this Ustilaginaceae, infecting plantlets only via the roots. Variability analysis of fungal isolates collected in different regions of France showed that some strains are specific to different lines. Cross infections are in agreement with the molecular data. These are the first published data of host-strain specificity on this pathosystem. This leads to propose an oligogenic gene resistance strategy for this model, as it has been developed for the interaction sorghum- Sporisorium reilianum reilianum

P120 A receptor kinase of the Lrk class is expressed at the maize basal endosperm transfer cell layer.

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The Lrk class is a group of receptor kinases described so far only in grasses. LRK10, a wheat member of this class, is encoded at the leaf rust resistance locus Lr10. Several other fungal disease resistance loci in grasses could also contain genes of the Lrk class. It is thus suggested that the Lrk receptor kinases are involved in some of the signal transduction pathways leading to plant resistance against pathogen infection. In the course of a search for genes expressed at the basal endosperm transfer cell layer of maize we have isolated a 2Kb cDNA clone encoding a serine/threonine receptor kinase of the Lrk class, ZmLrk2. The gene is located on the short arm of chromosome 8, close to the previously described ZmLrk1 pseudogene. ZmLrk2 mRNA can be detected in developing and germinating seeds, but also in roots and the aerial parts of the plant. "In situ" hybridisation confirms that in developing seeds the ZmLrk2 RNA accumulates at the endosperm transfer cells.

P121 Transgenic maize lines expressing a wheat Glu1-Dx5 high molecular-weight glutenin

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We have developed lines of transgenic maize containing the Glu1-Dx5 gene from wheat. Lines derived from four independent transformation events exhibit reduced pollen transmission of the transgene. We have also examined the inheritance of the bar transgene that was introduced by co-bombardment with the 1Dx5 transgene, and found that it co-segregates with the Glu1-Dx5 transgene, and exhibits reduced pollen transmission as well. Repeated cycles of selecting Glu1-Dx5 expressing kernels, planting them and self-pollinating the resulting plants has led to the development of lines that produce near-isogenic kernels, 50 % of which express the transgene and 50% of which do not. This allows us to determine the effect of the transgene on a trait by comparing kernels from the same ear. In this way, genotypic and environmental effects on comparisons between the expressing and non-expressing kernels are minimized. We have compared the nitrogen and carbon content of pairs of near-isogenic kernels and found that kernels producing the high-molecular weight glutenin contain about 40% more nitrogen.

P122 Functional analysis of the late pollen-specific MADS-box gene ZmMADS2
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The pollen grain is the male gametophyte of flowering plants. It originates during the process of microsporogenesis from a pollen mother cell (PMC) producing a tetrad of microspores through meiosis. During microgametogenesis microspores undergo a first mitotic division to form a large vegetative and a smaller generative cell. In maize, a further division of the generative cell takes place to generate two sperm cells within the trinucleate pollen grain. Upon germination, the vegetative cell forms the pollen tube, and the two sperm cells are transported to the embryo sac. So far, little is known about transcriptional regulation of these complex processes. We have isolated a MADS-box transcription factor gene (ZmMADS2) wich belongs to the group of late pollen genes (genes which are upregulated at very high levels during the last stages of pollen development). ZmMADS2 is highly upregulated shortly before anthesis and transcripts are translocated into the growing pollen tube upon germination. In order to study the function of ZmMADS2, we have isolated the corresponding genomic clone, which consists of eight exons and some 1.5 kbp upstream of the transcription start point, the latter probably representing the promoter. Promoter deletion constructs were tested in transient expression assays. Localization of the ZmMADS2 protein was analyzed by transgenic plants carrying a GFP-fusion protein under the control of the ZmMADS2 promoter. In addition, transgenic maize plants were generated carrying ZmMADS2 in antisense orientation. The results of these experiments will be presented.

P123 Expression of reporter genes in transgenic maize
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The mechanisms underlying the regulation of nutrient deposition in developing kernels remain essentially unsolved. In particular, we focus on zeins, the major storage protein in maize endosperm, their genes, their expression and regulation. As a starting point for the identification of factors affecting zein transcription, we have developed a genetic approach based on the stable introduction of a chimeric gene for green fluorescent protein (GFP) accumulation in maize endosperm. We used well-established protocols for maize transformation and regeneration of transgenic plants by particle bombardment of embryogenic callus. Several lines have been obtained that express the reporter gene at high levels, irrespective of generation and background. Seed viability and germination were unaffected by the transgene. More recently, plants with a translational fusion of a-zein/GFP genes have been generated. We have succeeded in detecting a stable protein in maize endosperm by western blot analysis. We expect that our reporter genes will be useful and easy-to-score markers in the search of genes controlling zein transcription and deposition.

P124 A Comparative Analysis of Genes Involved in Regulating the Chromatin Environment in the Genomes of Plants, Animals and Fungi. Selinger, David A.(1); Pandey, Ritu(1); Mount, David W.(1); Napoli, Carolyn(1); Bender, Judith(3); Richards, Eric(4); Jorgensen, Richard A.(1); Chandler, Vicki L.(1) (1) University of Arizona, Tucson, Arizona 85721, USA; (2) Johns Hopkins University, Baltimore, Maryland 21205, USA; (3) Washington University, St. Louis, Missouri 63130, USA

Remodeling the structure of chromatin and modification of histone and DNA molecules in chromatin by specific enzymes have major effects on gene expression and gene silencing. Genes that regulate gene expression through changes in chromatin structure and function have been identified in several model organisms. Using sequence similarity searches and these genes as queries, we have identified similar genes in the Arabidopsis genomic sequence and maize EST sequences (these genes can be found in the ChromDB database, accessible at http://ag.arizona.edu/chromatin/). We have identified more than 150 Arabidopsis genes and more than 70 maize genes that are likely to be involved in chromatin level gene control and have classified them based on characteristic conserved features and molecular phylogenies. A comparison of the plant genes with sequences from other eukaryotic genomes shows that one to one orthologous relationships exist for many proteins that are responsible for core functions of chromatin remodeling and histone acetylation. However, we have found several differences in plant chromatin genes when compared to the other genomes. For example, our comparisons of histone acetyl-transferases and deacetylases suggests that some of the diversification of these proteins occurred after the three main groups of eukaryotes diverged, giving rise to different complements of proteins in different lineages. Analysis of the HD2 class of proteins, which are unique to plants, indicates that duplication and divergence events have occurred since the divergence of monocots and dicots. In addition, the domain structures of some Arabidopsis ATP-dependent chromatin remodeling and histone acetyl-transferase genes differ from their cognates (orthologs) in yeast and metazoans. Several other Arabidopsis proteins lack domains, when compared to their orthologs, and others possess domains not found in orthologous proteins from fungi and animals.

P125 Functional Genomics of Maize Chromatin Level Gene Regulation. Chromatin Functional Genomics Consortium, (1,2,3,4,5,6) (1) University of Arizona, Tucson, Arizona 85721, USA ; (2) Johns Hopkins University, Baltimore, Maryland 21205, USA; (3) University of Missouri,Columbia, Missouri 65211, USA; (4) Purdue University, West Lafayette, Indiana 47907, USA; (5) Washington University, St. Louis, Missouri 63130, USA; (6) University of Wisconsin, Madison, Wisconsin 53706, USA

Using maize and Arabidopsis, we are producing tools and information necessary for a comprehensive study of the proteins and mechanisms responsible for gene regulation at the level of chromatin structure and function. The complete genome sequence of Arabidopsis has facilitated the identification of genes in maize. Comparison of conserved genes in two morphologically and physiologically different plants with different genome sizes and repetitive DNA content, will allow us to assess the functional generality of these genes. Our objective is to identify, mutate, and functionally analyze most of the several hundred genes in maize and Arabidopsis that contribute to chromatin-level control of gene expression. We have identified genes in the Arabidopsis genome sequence, in maize EST's and in EST's from other plants by sequence similarity to genes and protein domains known to be important in chromatin-level gene regulation. A summary of the genes identified can be found at http://ag.arizona.edu/chromatin/. We are producing dominant negative mutations for each targeted chromatin gene, using RNA silencing triggered by transgenes that produce double-stranded RNA homologous to target genes (Smith et al 2000; Nature 407, 319 - 320). As transformation is a key technology for this approach we are developing improved methods for transforming maize. Maize ESTs are being used as probes on Southern and Northern blots to screen for gene copy number and expression patterns. Nearly all ESTs screened so far hybridize to more than one band, suggesting that many chromatin genes in maize are duplicated. Genetic mapping is underway to determine chromosomal location of as many genes as possible. In addition, for each targeted gene, we are determining the complete double-strand sequence of the cDNA. Once mutants are available they will be subjected to a variety of biochemical and genetic assays to assess their role in gene regulation. Our progress on each of the above goals is described. Our initial results have yielded interesting observations on the complement of chromatin genes in the two plants compared to each other and to other organisms. In addition, the development of new mutational and transgenic technologies for maize will contribute to the study of other genes.

P126 Progress on Generating Uniform-Mu Seed Mutants
Settles, A. Mark(1); Baier, John(1); Latshaw, Sue(1); Koch, Karen(1); McCarty, Donald(1); Hannah, L. Curtis(1) (1) University of Florida, Gainesville, Florida 32611, USA

Plant genome sequencing is revealing a large number of genes with unknown functions or presumed functions based solely on sequence homology. To understand the roles of these genes, we need saturation forward genetics, because forward genetic strategies do not assume functional roles for a particular gene and can reveal novel pathways. We have begun a comprehensive dissection of the single locus seed mutants in maize. As part of this strategy, we have developed a transposon mutagenic inbred (Uniform-Mu) by backcrossing Robertson,s Mutator lines into color-converted W22. Our Uniform-Mu population has several essential features: 1) Active Mutator in an inbred background while maintaining mutation frequency. 2) Seed phenotypes are purged from the population each generation to ensure that the new mutations are independent events. 3) The pedigree of each line is known so that the immediate progenitor of every new mutation is known. 4) Genetic control over transposon activity to freeze transpositions once a new mutant is isolated. We have completed seven backcrosses and have currently generated 9,500 selfed lines from the backcross 5 (BC5) generation. Preliminary screens of these lines indicate that 5%-10% of the selfed plants segregate for single locus, recessive seed mutants. Our goal is to generate 2,000 independent mutants in order to saturate for multiple alleles of all seed phenotypes. This spring, we will generate an additional 20,000 BC5 selfs and a BC7 mutagenic population. In addition, each mutant we have already identified will be tested for heritability and Mu-inactive lines established for MuTAIL-PCR based cloning strategies.

P127 stc2: an orthologue of the volatile defense gene stc1
Shen, Binzhang(1); Lu, Dihui(1); Llaca, Victor(1); Messing, Joachim(1); Dooner, Hugo(1) (1) Rutgers University, Piscataway, New Jersey 08854, USA

stc1, the first sesquiterpene-cyclase gene in maize, is induced by a herbivore elicitor, volicitin, to produce a volatile-defense signal which may function in the attraction of herbivore natural enemies (BZ Shen, ZW Zheng and HK Dooner. 2000, PNAS, 97:14807-14812). In an attempt to understand the function and evolution of this new class of plant defense genes, we have embarked on the isolation and characterization of the stc1 orthologue. Using primers based on the stc1 coding sequence and template DNAs from the stc1 deletion mutants sh-bz:x2 and sh-bz:x3 (JP Mottinger, 1970, Genetics, 64:259-271), we have amplified stc1-related sequences. One of the PCR products, distinct in size from the stc1 but common in both mutants and the wild type, was cloned and sequenced. It shows homology to stc1 as well as other sesquiterpene-cyclase genes in the database and is designated stc2. stc2 is mapped to chromosome 6 using oat-maize addition lines and is about 4 cM from pl gene by a maize recombinant-inbred mapping population. Therefore, based on sequence homology and synteny, stc2 is the orthologue of stc1. The full-length stc2 gene has been isolated from a B73 BAC library. Finally, we will report a comparison of stc1 and stc2 in gene sequences and in patterns of transcript induction by insect elicitors.

P128 Expression of a tobacco MAPKKK gene, NPK1, confers salt tolerance in transgenic maize
Shou, Huixia (1); Frame, Bronwyn R. (1); Chiu, Wan-ling(2); Sheen, Jen(2); Wang, Kan(1) (1) Iowa State University, Ames, IA 50011-1010, USA; (2) Massachusetts General Hospital, Boston, MA 02114, USA

Expression of a tobacco MAPKKK gene, NPK1, confers salt tolerance in transgenic maize H. Shou, B. Frame, W. Chiu1, J. Sheen1, K. Wang Department of Agronomy, Iowa State University, Ames, IA 50011-1010 1Department of Molecular Biology, Massachusetts General Hospital, Boston, MA 02114 Nicotiana protein kinase, NPK1, is a mitogen-activated protein kinase kinase kinase (MAPKKK) involved in the transduction of the oxidative stress signal. The constitutively active NPK1 can substitute for H2O2 to induce specific stress-responsive genes and confers multiple stress tolerance in transgenic tobacco (Kovtun et al., 2000). To determine whether the oxidative-stress mediated MAPK cascade is conserved in monocots, the gene encoding the constitutively active NPK1 was introduced into maize using the biolistic gun-mediated immature zygotic embryo culture transformation system. Twelve independent transgenic maize events were produced. Southern blot analysis of the twelve transgenic events showed that they have different copy numbers and insertion patterns of the transgene. Copy numbers of the transgenic plants range from two to twenty per genome. RT-PCR results showed the existence of NPK1 transcript in all of the twelve lines. However, only three lines showed the NPK1 transcript by the Northern blot analysis, indicating that the NPK1 transgene expresses at different level in different transformation events. Significantly, salt tolerance experiments showed transgenic seeds have higher germination and growth rates than non-transformed control seeds in the high-salt containing medium. Kovtun, Y., Chiu, W-L., Tena, G., Sheen, J. (2000). Functional analysis of oxidative stress-activated mitogen-activated protein kinase cascade in plants. PNAS, 97:2940-2945.

P129 Transgenic plants allow further dissection of sequences required for paramutation in maize P1-RR gene.
Sidorenko, Lyudmila(1); Chandler, Vicki(1); Peterson, Thomas(2) (1) Department of Plants Sciences, University of Arizona; (2) Department of Zoology and Genetics, and Agronomy, Iowa State University

The maize P1-rr gene encodes a myb transcriptional activator conferring red pigmentation in husks, silks, cob and kernel pericarp. The upstream regulatory region of P1-rr spans about 6 kb of the upstream sequence and has a modular structure: basal promoter fragment Pb (-233 to +326), and two enhancer fragments, proximal P1.0 (-1252 to -234), and distal P1.2 (-6110 to -4842). Previous studies showed that P::GUS transgenes carrying the distal P1.2 enhancer fragment can induce paramutation of the endogenous P1-rr allele (Sidorenko and Peterson, Plant Cell, 2001). We are interested in fine dissection of the P1.2 fragment to identify the cis-acting elements responsible for P1-rr paramutation. To accomplish this goal we designed series of deletion constructs that span the P1.2 enhancer fragment. Transgenic plants were obtained for the first two deletions that separate P1.2 in two fragments of 405 bp (P0.4) and 864 bp (P0.8). Primary transgenic plants were outcrossed to naïve P1-rr and F1 progeny plants were examined for ear phenotypes. Results indicate that both tested independent events of P0.4b::GUS caused silencing of the endogenous P1-rr allele in all 12 crosses. The silenced phenotype, P1-rr', was observed in 72% of plants confirmed to carry the transgene. In contrast, no silencing was observed in crosses involving all three independent events of the P0.8b::GUS transgene. This indicates that sequences required to induce P1-rr' paramutation are located within the 405 bp fragment. Currently we are testing heritability and paramutagenicity of P1-rr' silenced by the P0.4 fragment. Results from other deletion constructs should allow further dissection of the sequences required for P1-rr paramutation.

P130 Characterization of the Aldehyde Dehydrogenase Gene Families of Zea mays and Arabidopsis
Skibbe, David S(1); Liu, Feng(1); Wen, Tsui-Jung(1); Cui, Xiangqin(1); Hsia, An-Ping(1); Yandeau, Marna(1); Fu, Yan(1); Simmons, Carl(2); Meeley, Robert B(2); Schnable, Patrick S(1) (1) Iowa State University, Ames, Iowa 50011, USA; (2) Pioneer Hi-Bred Intl., Johnston, Iowa 50131, USA

Cytoplasmic male sterility (cms) is characterized as a maternal inability to produce viable pollen. In T-cytoplasm, sterility arises from the premature degeneration of the tapetal cell layer during microspore development. This sterility can be overcome by the combined action of two nuclear restorer genes, rf1 and rf2. The rf2 gene encodes a mitochondrial aldehyde dehydrogenase (mtALDH; Cui et al., 1996, Science; Liu et al., Plant Cell, under revision). ALDHs have been widely studied in humans (for reviews see Lindahl, 1992; Yoshida et al., 1998), but relatively few studies have been conducted on the corresponding plant enzymes. Two complementary approaches, in vitro enzyme analyses and maize complementation experiments, are being used to study the roles of ALDHs in plants. Six ALDH genes from maize and Arabidopsis with similarity to rf2 (now termed rf2a) have been cloned and the encoded proteins biochemically characterized. The rf2b gene encodes an ALDH that is also thought to accumulate in the mitochondria, whereas the rf2c and rf2d genes encode ALDHs that are predicted to accumulate in the cytosol. In Arabidopsis, two putative mitochondrial and one putative cytosolic ALDH have been identified. In vitro enzyme assays on crude extracts of RF2A, RF2B, RF2C, RF2D, AtALDH1 and AtALDH2 proteins demonstrate that these ALDHs have somewhat different enzymatic properties. Preliminary transgenic analyses have demonstrated that a construct containing 5, and 3, rf2a non-coding sequence and the rf2a cDNA can complement the male sterile phenotype (i.e. restore fertility) in a cms-T rf2a/rf2a background. In addition, Mu transposon insertions have been identified in rf2c and rf2d using the Trait Utility System for Corn (TUSC). These candidate mutants represent a valuable tool for studying the roles of ALDHs in plants.

P131 Analysis of genes encoding Polycomb group proteins in maize
Springer, Nathan M.(1); Danilevskaya, Olga(2); Hermon, Pedro (2); Smith, Alan (1); Helentjaris, Tim(2); Phillips, Ronald L.(3); Kaeppler, Heidi F.(1); Kaeppler, Shawn M. (1) (1) Dept of Agronomy, University of Wisconsin-Madison, 1575 Linden Dr, Madison, Wisconsin 53706, USA.; (2) Pioneer Hi-Bred International, Inc., 7300 N. W. 62ndAve, Johnston, Iowa 50131, USA.; (3) Department of Agronomy and Plant Genetics, University of Minnesota, 1991 Buford Cir, St. Paul, Minnesota 55108, USA.

Polycomb group (PcG) proteins play an important role in developmental and epigenetic gene regulation in animals. Recent evidence has shown that PcG proteins in Arabidopsis are important for the regulation of plant development. We sought to characterize the PcG genes present in the maize genome. Homologues of three of the twelve cloned PcG genes were detected in maize. The cDNA sequences of three homologs of Enhancer of zeste (Mez1, Mez2, and Mez3) and two homologs of extra sex combs (Mesc1 and Mesc2) were obtained. In addition, a sequence with homology to Enhancer of Polycomb was found in maize and Arabidopsis. The expression pattern of each of these genes was analyzed by RT-PCR. Mesc1 transcripts were detected throughout the plant while Mesc2 transcripts were only present in the developing seed. The domain composition of the plant PcG proteins was analyzed and revealed regions of conservation between plants and animals as well as regions specifically conserved in the plant PcG proteins. The relationships of the maize proteins to plant and animal homologs are discussed.

P132 Mitochondrial RNA Polymerase: Reverse Genetics and Biochemistry
Boisson, Murielle(1); Caoile, Angel(1); Stern, David(1); Chang, Ching-Chun(1); Lerbs-Mache, Silva(2); Meeley, Robert(3) (1) Boyce Thompson Inst., Cornell Univ., Ithaca NY 14853; (2) Universite Josef-Fourier, Grenoble, France; (3) Pioneer Hi-Bred International

see TALK ABSTRACT # T16

SESSION 4

ORGANELLES/PLANT DEFENSE

SATURDAY MORNING

P133 DAM-mediated male-sterility is reversed by removal of an adjacent [35S] element using FLP recombinase.
Unger, Erica (1); Cigan, A. Mark(1); Xu, Rui-ji(1); Haug Collet, Kristin(1); Kendall, Tim(1); Peterson, Dave(2) (1) Plant Reproductive Biology, Pioneer Hi-Bred, Johnston, IA 50131, USA; (2) Gene Targeting Group, Pioneer Hi-Bred, Johnston, IA 50131, USA

The male fertility phenotype associated with anther-targeted expression of E. coli DNA (Adenosine-N6-)-Methyltransferase (DAM) by the maize promoter 5126 (5126:DAM) has been shown to be dependent upon the adjacent selectable marker gene; linking 5126:DAM to 35S:PAT resulted in a high frequency of male sterile plants with anthers severely reduced in size, while linking the 5126:DAM gene to the Ubi:PAT gene resuled in a high frequency of male fertile plants. In addition, factors such as gene orientation and regulatory sequences mediating expression of an adjacent gene have been shown to influence DAM-mediated sterililty in maize. Constructs that place the 35S enhancer between the 5126:DAM and Ubi:PAT genes resulted in a high frequency of male-sterile plants with reduced anther size while PAT gene transcript levels consistently increased when regions from the 35S promoter were included, suggesting that PAT gene transcription may contribute to the 5126:DAM[35S]UBI:PAT sterility phenotype. Using this DAM-mediated phenotype, experiments employing FLP-mediated recombination were designed to evaluate the efficiency of excision and to test the contribution of the 35S element in this context. Various 5126:DAM[35S]UBI:PAT constructs were generated to include FLP recombination target (FRT) sequences that allowed for removal of all or part of the [35S]UBI:PAT transcription unit in the presence of constitutively expressed FLP while evaluating the impact on male fertility. Removal of [35S]UBI:PAT by FLP-mediated excision restored fertility to plants containing 5126:DAM, while 5126:DAM[35S] plants generated following excision of only UBI:PAT remained male-sterile. In addition, removal of only the [35S] element from plants containing 5126:DAM[35S]UBI:PAT resulted not only in the restoration of fertility but also in a significant reduction in PAT expression. Chimeric PAT- and DAM-mediated phenotypes were observed in several events, suggesting that the efficiency of FLP-mediated excision can vary. Southern analysis correlated these abberent phenotypes with incomplete excision. Although the factors that contribute to the efficiency of site-specific excision in maize have not been defined, these experiments provided an opportunity to examine the contribution of adjacent gene components at unique integration sites within the maize genome.

P134 SCAR markers for the Ht1, Ht2, Ht3 and Htn1 resistance genes in maize.
van Staden, Derick (D.)(1); Lambert, Carol-Ann (CA.)(1); Lehmensiek, Anke (A.)(1); Retief, Anries (A.E.)(1) (1) University of Stellenbosch, Stellenbosch 7602, RSA

Amplified fragment length polymorphisms (AFLPs) were used on two NIL pairs each of Ht1, Ht2, Ht3 and Htn1 to identify common polymorphic fragments in resistant/susceptible lines. Polymorphic fragments were converted to sequenced characterized amplified region (SCAR) markers and were mapped using two commercially available recombinant inbred line (RIL) populations. Four polymorphic SCAR markers were identified for Ht1 and three of these mapped to chromosome 2.07. Two SCAR markers were polymorphic in the Ht2 NILs and mapped to chromosome 8.05/06. For the Ht3 NIL pairs, two SCAR markers and a microsatellite marker (bnlg1666) were found to be polymorphic. One of the SCAR markers and the microsatellite marker were mapped to chromosome 7.04. This is the first tentative mapping position for the Ht3 locus. Seven SCAR markers were developed for the Htn1 locus, five of which were mapped to one region of maize chromosome 8.05/06. The simple PCR markers which were developed for Ht1, Ht2, Ht3 and Htn1 can be used in gene identification, fine mapping and marker assisted selection.

P135 Agrobacterium-mediated transformation of maize Hi II immature zygotic embryos using a simple binary vector system
Frame, Bronwyn R.(1); Zhang, Zhanyuan(1); Xiang, Chengbin(1); Chikwamba, Rachel K.(1); Shou, Huixia(1); Fonger, Tina (1); Pegg, Sue-Ellen(1); Wang, Kan(1) (1) Iowa State University, Ames, IA 50011-1010, USA

see ABSTRACT# 11

MAIZE GENOME WORKSHOP

SATURDAY PM

P136 Evaluation of Computational Approaches for Gene Discovery in Maize
Yao, Hong(1); Wen, Tsui-Jung(1); Skibbe, Dave(1); Cui, Xiangqin(1); Cao, Jun(1); Liu, Feng(1); Dietrich, Charles(1); Fu, Yan(1); Li, Jin(1); Smith, Heather(1); Nikolau, Basil J.(1); Schnable, Patrick S.(1) (1) Iowa State University, Ames, Iowa 50011, USA

Seven algorithms for gene discovery were evaluated using a data set that consists of 8 previously unreleased maize genes (*gl8a*, *pdc2*, *pdc3*, *rf2b*, *rf2c*, *rf2d*, *rf2e1*, *rth1*). In total, these validated gene structures contain 71 exons and 63 introns that were not part of the training sets used in the development of any of the algorithms. The algorithms assessed include SplicePredictor, NetGene 2, GENSCAN, FGENEP, FGENESH, GeneMark.hmm and GeneSeqer. The accuracy of splice site predictions and gene structure predictions were evaluated for each algorithm. The influence on gene prediction accuracy of such factors as GC content, exon length and number, and intron length and number were investigated. These algorithms were also used to identify two predicted genes (*yz1* and *x1*) in the *a1-sh2* interval. These predicted genes were subsequently validated via RT-PCR and sequence analysis of cDNA clones.

P137 AFLP Mapping of Insect Resistance in Maize
Bauer, Matthew J(1); Musket, Theresa A(1); Davis, Georgia L(1) (1) University of Missouri - Columbia

In the United States, fall armyworm (*Spodoptera frugiperda* J.E. Smith) and southwestern corn borer (*Diatraea grandiosella* Dyar), can cause a severe reduction in crop yields. Both of these Lepidopteran insects have different life cycles and host ranges. The fall armyworm host range includes 60 plant species while southwestern corn borer has a host range of three plant species. Non-transgenic elite maize germplasm is susceptible to these pests. A known insect resitant maize line, Mp705, was used to identify a quanitative trait locus (QTL) on chromosome 9. This QTL region was coincident with the *Glossy15 (Gl15)* gene. *Gl15* is a transcription factor that is involved in the timing of the juvenile-to-adult phase change. The resistant parent allele of *Gl15* gives resistant plants more adult leaves than susceptible plants. Two other QTL regions have also been identified with no known function. Our goal is to identify and characterize Lepidopteran resistance genes in maize. Our specific objectives are to create an amplified fragment length polymorphism (AFLP) map that is integrated with the prior restriction fragment length polymorphism (RFLP) map and to select three primers that can be used to screen backcross progeny for rapid introgression of a resistance QTL. AFLP,s involve cutting genomic DNA with restriction enzymes, and ligating adaptors to those fragments. The adaptors and the adjacent restriction site serve as a priming site for amplification. These DNA fragments become a templete for PCR. The amplified fragments are then analyzed by gel electrophoresis. AFLP,s are cheaper, and faster than most conventional marker technologies.

P138 The Evolutive Cycle Of Maize Inbreds Developed In Argentina

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During 1990, a maize breeding programme was initiated aimed to obtain waxy endosperm maize commercial hybrids capable of being used by several industries. It is also included the development of high quality protein maize hybrids either for animal and human feeding. Evolutive cycle traits were measured in the foundational materials, backcrosses, inbreds and different kind of hybrids derived from them considering not only the number of days but also the heat units neccesary for mid-tasseling, mid-pollen shedding and mid-silking. Our conclusions were based on the heat units neccesary for mid silking (R1 stage according to Hanway scale), as this let us compare amongst genotypes independently from environment factors. Most of the waxy maizes selected as foundationals were precocious (heat units required for silking: 418°C-518°C). On the other hand, opaque-2 foundationals showed a long evolutive cycle and needed 750°C to 870°C. Heat units requirements of the normal endosperm maizes also used ranged from 484°C to 568°C, by which their cycle is considered short to medium. Data collected from 1990-to-date, points that whether inbreeding lenghtens the evolutive cycle as more heat units are required for silking independently from the number of days (a badly outlined variable by the environment), new high quality protein S1-S4 inbreds requiring only 472°C to 535°C for silking were developed, which confirms that selection for precocity in these materials was full effective. Likewise, waxy endosperm S3-S7 inbreds fullfill their heat requirements with 441°C to 551°C and normal endosperm S2-S4 inbreds do the same ranking from 490°C to 544°C. In experimental hybrids and backcrosses obtained using some of the most advanced inbreds developed, it was seen that the evolutive cycle length is overdominated by the most precocious parent of the crossing. The results let us conclude that the different inbreds developed or under developing process have a short or medium evolutive cycle length, which would benefit the growing of their hybrids in south-east and western regions of the province of Buenos Aires in Argentina.

P139 QTL and NIL analysis of maize kernel composition and tassel architecture

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High oil corn (HOC) is a more efficient feed for livestock than traditional corn and is now being grown in the Midwest. The purpose of this research is to map quantitative trait loci (QTL) for the kernel traits, protein, oil, and starch, in an effort to better understand their chromosomal location and function. A BC1S4 mapping population was created from the parents B73 and Illinois High Oil (IHO). 111 SSR markers were placed on the population and subsequent QTL analysis was performed on the population per se as well as testcrosses. QTL for oil were revealed on chromosomes 1, 6, 7, 8, and 10. Sets of near-isogenic lines (NILs) have been developed and have confirmed the existence of a major oil QTL on chromosome 6, and more precisely defined this region. In one set of these NILs the QTL accounts for 1-2 percent of oil. These genetic materials will be useful for fine mapping and eventual cloning of individual high oil genes. Tassel morphology is relevant to the TopCross high oil pollination system and hybrid seed production. We have reported previously on QTL for tassel branch number, angle, and tassel weight and will present additional information on these traits. We have recently started measuring spikelet density and present initial results that indicate a QTL for spikelet density is linked to the npi449 marker in bin 5.04 which contains thick tassel dwarf1 (td1).

P140 Role of Sh2 gene in both maize kernel diversity and maize domestication
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A major source of starch for both food and industry is the cereals, especially maize and wheat. In maize, starch accounts for about 80% of kernel weight. ADP-glucose pyrophosphorylase (AGPase) is a key enzyme in the early steps of starch biosynthesis. The endosperm AGPase is a heterotetramer consisting of two similar but distinct subunits, encoded by Sh2 and Bt2 genes. Several studies showed that 1) Sh2 colocalised with kernel trait QTLs in several populations and 2) sh2 mutants are strongly starch deficient. Thus Sh2 can be considered as a candidate gene for starch content and seed weight in cultivated maize. To confirm this hypothesis, a large population of cultivated inbred lines was characterised for both kernel traits (seed weight, starch content, AGPase activity, etc) and Sh2 molecular polymorphisms (SNP). Statistical associations between these phenotypic and molecular polymorphisms were tested. The study population was composed of unrelated lines, exhibiting low gametic desequilibrium, in order to avoid misleading associations. Our molecular study was extended to wild relatives of maize (mexicana, parviglumis and huehuetenanguensis subspecies). Phylogenetic approach was performed to determine whether Sh2 has been a target of selection throughout domestication.

P141 Quantitative trait locus analysis of rind penetrometer resistance in four maize populations
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Stalk lodging in maize (Zea mays L.) causes losses estimated to range from 5 to 20% annually in the United States. Selection for rind penetrometer resistance (RPR) has proven useful in enhancing germplasm for stalk strength, and therefore improving stalk lodging resistance. We conducted quantitative trait locus (QTL) analyses for rind penetrometer resistance (RPR) in four F2:3 populations. The populations were constructed using MoSCSSS-High (selection for high RPR), MoSCSSS-Low (selection for low RPR), MoSQB-Low (selection for low stalk crushing strength), Mo47, and B73. Simple sequence repeat (SSR) and restriction fragment length polymorphism (RFLP) markers were used to genotype individuals in each population and data was collected for RPR, plant height, and ear height. Means combined over locations were used to conduct composite interval mapping using QTL Cartographer. Ten, thirteen, eight, and nine QTL were detected for RPR in the four populations and accounted for 60.3%, 70.3%, 41.9%, and 76.8% of the phenotypic variation, respectively. Only one QTL was in common among all four populations, while eight QTL were in common between two of the four populations. Differences in the QTL detected among the populations may be explained by lack of variation at a QTL between parents of a population, epistasis, or a combination the two. Possible candidate genes include bm1 and bm3 involved in lignin content, and gl15 which has been implicated in timing of vegetative phase change.

P142 AFLP Mapping for Aflatoxin Reduction in Maize
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Aflatoxin contamination of corn grain has been a problem in recent years. This mycotoxin is a secondary metabolite of some strains of *Aspergillus flavus*. If long term exposure occurs at low levels, aflatoxin can be a potent carcinogen but if short term, high dose exposure occures, it may lead to toxicosis. The FDA has set 20 ppb as the maximum allowable level in a lot of corn. This often leads to economic loss for the producers. Cotton, rice and peanut crops are also affected by aflatoxin. Primary factors for aflatoxin production are the correct genes in the plant and the fungus and the correct developmental stage of the plant. Secondary factors that determine the level of toxin in the grain are heat and drought conditions. Elite germplasm can accumulate high levels of the toxin. Mp313E, a tropically derived inbred, has low toxin accumulation potential. Eight aflatoxin reduction quantitative trait loci (QTL) were identified in an F2:F3 population of Mp313E (resistant) x Va35 (susceptible) including a major resistance factor on chromosome 4. Our goal is to identify and characterize the QTL, *qafr1*, on chromosome 4. Our specific objectives are to create a map integrating new amplified fragment length polymorphism (AFLP) data with the existing restriction fragment length poylmorphism (RFLP) data and to select a subset of three primers to survey the genome for rapid screening of backcross and recombinant progeny. AFLPs are a PCR-based technology that can be used to obtain genotypic information on a large number of loci simultaneously. This technique uses restriction enzymes and selective adapters, or amplification primers under stringent PCR conditions to produce DNA fingerprints that can be used for genetic analysis.

P143 The genetics of chlorogenic acid and flavone synthesis in the maize inbred Mo6
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Chlorogenic acid, a phenylpropanoid compound, and the flavones maysin and isoorientin have been shown to contribute to corn earworm (Helicopvera zea, Boddie) resistance when produced in the silks of maize (Zea mays L.). We examined the F2 offspring from a cross of two inbred lines (Mo6 x A619) to identify loci affecting accumulation of these chemicals. These inbred lines were chosen due to their clear differences in compound accumulation. Mo6 accumulates an unusually high level of chlorogenic acid, with negligible amounts produced in A619. We constructed a linkage map using 93 simple sequence repeat markers spanning all ten chromosomes to associate differences in phenotype with genetic loci. Using composite interval mapping as implemented in QTLCARTOGRAPHER, several significant loci were identified. Our results indicate loci associated with the production of all three compounds on chromosome 1 near marker locus bnlg182 and on chromosome 2 near bnlg1138. On chromosome 5, loci for both maysin and chlorogenic acid are located near marker loci bnlg1346 and mmc0282, respectively. Additional loci for chlorogenic acid are located on chromosome 3 (bnlg1113), chromosome 4 (bnlg1755 and umc1173) and chromosome 8 (umc1309). Analysis of the roles and interactions of these loci and the identification of candidate genes will provide a clearer definition of flavone and chlorogenic acid synthesis.

P144 Linkage of molecular markers to Cercospora zeae-maydis resistance QTL via selective genotyping

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Gray leaf spot, caused by the foliar pathogen, Cercospora zeae-maydis, can greatly reduce grain yield in conducive environments worldwide. Resistance to C. zeae-maydis is quantitative in nature (Bubeck et al, 1993; Saghai-Maroof et al, 1996; Clements et al, 2000). In this study, we report a new source of resistance and evaluate its genetic basis using molecular markers. The resistant inbred, Vo613Y, was crossed to the susceptible inbred, Pa405, to obtain a population of 144 F2:3 families. These families were evaluated in Ohio, USA, and Cedara, RSA, for resistance to C. zeae-maydis based on a percent leaf area affected (PLAA) (Smith, 1989) visual score of the ear-leaf of five plants per plot. The 20 most resistant and susceptible progeny of the population, along with 20 intermediate lines were self-pollinated to obtain F3:4 lines which were evaluated at two locations in Ohio in 1999 and 2000, and used for genotyping with RFLP and SSR molecular markers. Data were analyzed based on F2:3 and F3:4 means over all environments using one-way ANOVA and Mapmaker/QTL. QTLs located on two chromosomes (1L and 2L) explained 14% and 15% of the variance, respectively, across both generations and in all environments tested. A Spearman rank correlation of the 144 F2:3 families evaluated in Ohio and Cedara showed that genotypes retained their relative ranks across macro environments. These results indicate that Vo613Y is a source of resistance that can be deployed effectively in both Africa and the US.

P145 Quantitative trait loci associated with cell wall components and resistance to the European corn borer in maize

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European corn borer (ECB) is a major pest of temperate maize, with yield losses and control measures costing over one billion dollars annually. The objectives of this study were to analyze an F3 population of B73 (susceptible to ECB stalk tunneling, low levels of NDF, ADF, and lignin in the stalk and sheath) x DE811 (resistant, high NDF, ADF and lignin) for 1) QTL associated with resistance to ECB and 2) QTL associated with NDF, ADF, and lignin in the stalk and sheath tissues and 3) to determine whether the QTL for NDF, ADF, and lignin are associated with those detected for ECB resistance. Seven QTL for resistance to ECB stalk tunneling were found on chromosomes 1, 3, 4, 5, and 8, with two QTL each on chromosomes 1 and 5. The analyses for neutral and acid detergent fiber (NDF, the hemicellulose, cellulose, and lignin fractions of the cell wall; ADF, the cellulose and lignin fractions) and lignin were performed on stalk and leaf sheath tissue sampled from the same environments. QTL for stalk NDF, ADF and lignin were detected on chromosomes 1, 2, 3, 4, 6, 7 and 9. QTL for sheath NDF and ADF were detected on chromosomes 1, 3, 4, 7, 8, 9, and 10. Four of the seven QTL detected for resistance to ECB tunneling are linked to QTL for NDF, ADF and lignin in the stalk and sheath tissue. Several candidate genes are located in the same chromosomal regions as QTL for NDF, ADF, and lignin. Analysis of the same traits on a population of recombinant inbred lines (RILs) derived from the same parents is currently underway.

P146 A High-Throughput system For Screening Backcross Progeny For Resistance Alleles
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Aspergillus flavus is a soil born fungus that infects numerous crop plants such as corn, rice, cotton, fruits, and nuts. It produces aflatoxin, a carcinogen that contaminates food and feed. Studies have shown an association between aflatoxin and primary liver cancer in human and certain animal systems. A quantitative trait locus (QTL) for aflatoxin reduction, *qafr1*, Mp313E was identified on chromosome 4. This gene accounts for more than 35% of the reduction in aflatoxin in our mapping population. Our goal is to reduce or remove a major threat of aflatoxin contamination in maize by identifying the genes that might reduce aflatoxin accumulation using simple sequence repeats (SSR) and amplified fragment polymorphisms (AFLP) markers. AFLP is a powerful DNA fingerprinting technique for DNAs of any origin or complexity. It is based on the selective PCR amplification of restriction fragments from a total digest of genomic DNA. This technique uses restriction of DNA, amplification of sets of restriction fragments, with selective oligonucleotide adapters, and gel analysis. Our objective is to develop a group of markers to evaluate BC1 and BC2 individuals using SSR and AFLP markers, and to identify individuals from these materials to use in cloning our resistance gene. Fifty SSR primer pairs from the chromosome 4 aflatoxin QTL region will be screened to identify polymorphic markers that can be used to screen for presence of the resistant parent allele in backcross and recombinant progeny. A subset of three AFLP primer pairs were selected based on even marker distribution to cover the genome. The primer pairs were amplified individually and multiplexed for analysis on the ABI3700. Backcross and recombinant progeny were screened to identify individuals with the *qafr1* allele from Mp313E and the maximum percentage of Va35 (susceptible parent) alleles in the remainder of the genome.

P147 Genetic Components of Photoperiod Response in Maize
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Photoperiod response affects the transition from vegetative to reproductive phase and limits the evaluation and use of tropical germplasm in maize breeding programs. Understanding its genetic control should facilitate the exchange of germplasm between breeding programs in temperate and tropical latitudes. The number of days from planting to anthesis (AD) was studied to assess photoperiod sensitivity in 236 F3 lines derived from a cross between photoperiod-sensitive inbred CML9 and photoperiod-insensitive inbred A632Ht. The F3 lines were evaluated under long-days and short-days in 1995, 1996 and 1997 at Tlaltizapan, Mexico and Ames, Iowa. QTL analysis, facilitated by a genetic map of 128 RFLP and SSR loci, was conducted for each photoperiod using combined and individual environments. A unique set of QTL was detected in each photoperiod. QTL on chromosome 2 had similar positions in both photoperiods. QTL on chromosome 9 had close map position in both photoperiods but contrasting parental effects suggested two different QTL on that chromosome. QTL on chromosomes 3 (npi108a), 8, 10 and 9 (umc39d) had the strongest association with AD under long-days. QTL on chromosomes 1, 3 (umc102), 4 and 9 (umc81) had the strongest association with AD in short-day environments. CML9 alleles increased AD at most QTL. Dominance deviations were mainly towards lower values of AD. In long-day environments, one epistatic interaction was observed between chromosomes 3 and 4. Candidate genes id1 and phy1, phyA2 and phyB2 were linked with QTL. Analyses of photoperiod response based on morphological traits of the same population and with RILs are being conducted.

P148 Genetic Analysis of Phosphorus Uptake in Maize

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Efficient use of mineral resources is critical to the long-term sustainability of U.S. and world agriculture. Phosphorus is the most limiting nutrient for plant growth in the world, particularly in weathered soils of the tropics. Genetic mapping of chromosome regions associated with maize growth under phosphorus limiting soil conditions has been conducted in this experiment. Quantitative trait loci (QTL) for shoot weight, days to seedling emergence, flowering date, and plant height have been identified using recombinant inbred lines planted in research plots with high and low phosphorus levels. Previously identified QTL for shoot weight of the recombinant inbred lines grown in the greenhouse under low phosphorus soil conditions appear to overlap with several of the QTL identified for shoot weight in the field. The QTL for shoot weight under high phosphorus in the field also appear to be in the same chromosomal regions as those identified for shoot weight under low phosphorus. Genetic mapping results and relationships between plant growth responses will be presented.

P149 Genetic Analyses and Marker Assisted Backcrossing of Resistance to Aflatoxin Production in Maize

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Aflatoxin B1 is one of the mycotoxins produced by Aspergillus flavus Fr: Link, which is an extremely potent, naturally occurring carcinogen. Contamination of corn grain with aflatoxins can be an economically important problem in maize. Considerable genotype x environment interactions for aflatoxin production make breeding for resistance and genetic analysis very difficult. Evaluation of resistance sources using diallel analysis from 1995 to 1998 confirmed Tex6 as a good source of resistance and revealed that Oh516 is a promising new source of resistance, that is complementary to Tex6. Two mapping populations derived from Tex6 were studied. Tex6 x B73 F2:3 and Tex6 backcross to B73 BC1S1 populations were inoculated and evaluated in 1996 and 1997 for aflatoxin levels. Markers associated with a QTL for resistance were detected on chromosome 5L in both populations. In 1997, this QTL region explained 25% and 8% of the total phenotypic variation in BC1S1 and F2:3 populations, respectively. Year 1996 was not conducive to expression of adequate levels of aflatoxin among the families, and we did not detect any QTL. Marker assisted backcrossing is being performed for the QTL on 4L in Mp313E identified by G. Davis and coworkers. We genotyped BC1 plants and backcrossed selected plants to FR1064 to develop BC2 plants, and these have been backcrossed again to FR1064 in Hawaii to develop BC3 seed. We have backcrossed plants with chromosomal region 5L from Tex6 in BC1S1 families to B73 to develop BC2 seed.

P150 Integrating AFLP Markers into an Existing RFLP Map for the Sugary Enhancer1 (se1) Trait in Maize

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The overall goal of this project is to provide a clearer understanding of the genetic and biosynthetic mechanisms involved in the accumulation of sugars in the recessive mutant phenotype sugary enhancer1 (se1) in maize. The molecular basis for this mutation is unknown. This endosperm carbohydrate mutation when homozygous in a sugary1 (su1) background has been observed to increase sugar content by 60-100%, improving consumer desirability while extending post-harvest maintenance of eating quality. An important application of this research is to provide DNA markers by which sweet corn breeders and researchers can more reliably discern the se1 genotype, thereby leading to greater commercial utilization. One aspect of this project seeks to assess the usefulness of AFLPs in improving the resolution of a previous linkage map based on RFLPs, and suggest new markers more tightly linked to loci associated with the se1 phenotype. The population studied is based on the inbreds W6786 (su1; Se1) X IL731a (su1; se1). Sugar levels were measured in 214 F2:3 families, and RFLPs were used to generate a linkage map. The results showed RFLPs associated with loci contributing to kernel sugar content on chromosomes 1-6. A major locus contributing to the se1 phenotype was located on the long arm of chromosome 2, 12.1cM from the marker umc36a. AFLPs have been chosen as a potential source of new markers closer to this major locus, and contributing minor loci. Fluorescently labeled AFLP primers from Applied Biosystems were screened on the parents of the population and primer combinations were chosen which gave the highest number of clear polymorphisms. These primer combinations were applied to the entire population. The results were combined with the RFLP and phenotypic information, and results are presented.

P151 Analysis of nutritionally limiting amino acids in maize populations

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The nutritional value of corn is limited by the level of lysine, tryptophan and methionine in the kernel. Determination of the levels of these amino acids normally done by HPLC and costs around $100 per sample. In plant breeding programs, it is necessary to analyze thousands of samples, so it is prohibitively expensive to use amino acid analysis to select for varieties with increased levels of these amino acids. Instead, breeders have relied on mutation breeding and more recently, transgenic approaches to increase the levels of these amino acids in maize. Here I report the development of high-throughput methods to quantify these amino acids, using either chemical analysis or auxotrophic mutants of E. coli. The cost of these analyses is less than $1 per sample, which is sufficiently low to meet the high-throughout needs of a breeding program. The amino acid content of several maize populations as well as five of inbred lines and their o2 conversions has been analyzed. Individuals from these populations have been identified with tryptophan levels that are competitive with those of o2 lines. It may therefore be possible to develop populations and inbred lines with tryptophan levels equivalent to o2 varieties without the detrimental affects of the o2 mutation.

P152 Nucleotide Diversity and Association Testing of Putative Maize Flowering Time Genes
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Flowering time is an agronomically important quantitative trait, which is potentially influenced by a large number of maize genes. Association testing, using inbred lines, provides a rapid means of dissecting the effects of these putative flowering time genes at very high resolution, provided that the effects of population structure are controlled in the statistical analysis. Five positional candidate genes, believed to be involved in flowering time, have been studied: dwarf3 (d3), dwarf8 (d8), indeterminate1 (id1), phytochromeB (phyB), and teosinte branched1 (tb1). Each candidate gene was PCR amplified and sequenced from at least 32 maize inbred lines. Association tests were used to assess the effect of sequence polymorphisms on phenotypic variation. Statistical methods of controlling population structure (Pritchard et al., 2000) were adapted for use with quantitative traits, to eliminate spurious associations. The results of these association tests, population structure, linkage disequilibrium, and nucleotide diversity will be discussed for each of these genes. Understanding the effects of such nucleotide polymorphisms and their presence in the germplasm will improve selective breeding techniques for these quantitative traits.

P153 Use of recombinant inbreds to identify QTL controlling genetic recombination in maize
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Previously (Weber et al. 1999, 41st Maize Conference Proceedings, p24), we compared recombination frequencies in 11 pairs of parents of recombinant inbred lines (RIs) that had been or were being mapped using molecular markers. Each parent was crossed with inbred testers that possessed linked morphological markers on chromosome 5 (a2 bt1 pr1), 7 (o2 v5 gl1) or 9 (yg2 sh1 wx1). The F1s were reciprocally testcrossed, and the testcross ears were classified to determine recombination frequencies associated with these lines. We found that the parents of one set of RIs (Hi31 and Ki14) from Brewbaker,s laboratory displayed the greatest recombinational differences. RIs from these parents were generated by Brewbaker,s group and were mapped at 127 RFLP loci by Ming and McMullen. The RIs from these parents that had already been mapped using molecular markers were provided by Brewbaker,s laboratory. Each of the RIs was crossed to the extent possible with the inbred testers for chromosomes 5, 7, and 9. The F1s were testcrossed, and the progeny produced are being classified. This report describes the analysis of the a2-bt and bt-pr regions on chromosome 5. Using Qgene and 91 RIs from these parents, we identified a QTL that impacts recombination in the a2-bt interval on chromosome 5 that is near the a2-bt1 interval. This QTL could be a gene(s) that impacts recombination in this region or the recombinational difference could be due to structural heterozygosity in this region in the Hi31 and Ki14 parents. We also analyzed recombination in the bt1-pr1 region in 47 RIs from these parents and did not detect any QTLs that affect recombination. Fewer RIs could be analyzed for this interval because the Ki14 parent was pr/pr and only about half of the RIs contained the dominant allele of this locus. We are continuing this analysis for the other chromosomes.

P154 Diversity and Association Studies in the Kernel Starch Genes Sugary1, Shrunken1, Shrunken2, and Brittle2

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Sugary1 (Su1), Shrunken1 (Sh1), Shrunken2 (Sh2), and Brittle2 (Bt2) all encode enzymes involved in the production of starch in the maize kernel. These genes have been PCR amplified and sequenced in a diverse set of 32 maize lines. Interesting polymorphisms found in all 4 starch candidate genes were used in association tests that correlate differences in sequence data with differences in phenotype seen between maize lines. The SNP responsible for the production of sweet corn was identified in Su1. Overall diversity of Su1 was very low (P = 0.27%), while Z. mays ssp. parviglumis showed much higher diversity than did the 32 maize lines. This finding lead us to believe that the Su1 gene played a key role in the domestication of maize. Sh1 is a moderately diverse gene (P = 1.1%). One region of the gene exhibited purifying selection, while an adjacent area was under balancing selection. Patterns of linkage disequilibrium (LD) indicated LD in Sh1 falls off quickly within 1 kb, while Su1 shows LD can extend up to 10 kb. Our study emphasizes the essential task of sequencing from many accessions to elucidate interesting and informative alleles within a candidate gene.

P156 High Throughput Transposon Mutagenesis in Maize

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Transposon insertion mutagenesis is a powerful tool for developing an understanding of cereal gene function. We have previously shown that the MuAFLP method is both efficient and highly reproducible when amplifying Mutator transposon insertion flanking sequences from individual plants, and that these flanking sequences represent germinal insertion events that have occurred preferentially within and around coding sequence. Through using amplified Mu flanking sequences as a mixed hybridisation probe, we identified homologous sequence within arrays of cDNAs. Our results demonstrate that this method is suitable for the identification of insertion events within large numbers of genes expressed during specific stages of maize development. Alternatively, through arraying amplified Mu flanking sequences from individual plants, followed by hybridisation with labelled cDNAs, we can rapidly identify plants harbouring insertion events within specific genes. The combination of these procedures allows the application of high throughput mutagenesis to further our understanding specific developmental processes in maize.

P157 A genome-wide analysis of MITE multimers in rice (O. sativa cv Nipponbare)
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A 128-bp insertion into the maize waxy-B2 allele led to the discovery of Tourist, a family of miniature inverted repeat transposable elements (MITEs). Some Tourist elements are present as tandem repeats or multimers, composed of a variety of nested and distantly related MITEs. Multimers are not observed for other maize MITE families. To test whether the formation of mutlimers is a common feature of MITEs, a more thorough survey, including an estimation of the proportion of the multimers, was performed with 25 Mb of publicly available rice genome sequence. Among the 5700 MITEs identified, over 10% are present as multimers. Interestingly, the proportions of multimers differ for different MITE families. Furthermore, high self-insertion frequencies suggest that some MITEs preferentially target members of the same family. That all 300 multimers are unique, indicates that multimers are not capable of further amplification. Finally, the genome-wide analysis of multimers in rice has provided a novel way to determine the relative timing of amplification of different families of transposable elements.

P158 Dasheng: a novel non-autonomous LTR retro-element that targets condensed chromatin
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Dasheng element was first discovered as a group of highly homologous sequences in the CUGI (Clemson University Genome Institute) rice (O. sativa cv Nipponbare) BAC end database. Subsequent searches revealed that the sequence of Dasheng contains all the putative cis-elements of LTR retrotransposons, but the internal region does not contain any of the coding regions that are associated with retrotransposons. For this reason it is believed to be non-autonomous. Most of the 1000 to 2000 copies appear to be full-length elements with very high sequence identity. Using the transposon display technique, 215 elements were mapped to all twelve rice chromosomes using a doubled haploid mapping population derived from a cross between IR64 (indica) and Azucena (japonica). Half of the elements are clustered in regions that account for only 4% of the total mapping distance and all clusters, except one, are located in centromeric and pericentromeric regions. Furthermore, there appears to be a strong correlation between the position of Dasheng clusters and highly condensed regions of the chromosome. The distribution pattern was further confirmed by FISH analysis, which showed that most of the elements are associated with the heterchromatin around the centromeric regions. In addition, about 65% of the complete Dasheng elements (11 out of 17) in the database show over 99.5% homology between the two LTRs, suggesting the recent amplification of this family. Taken together, Dasheng is a non-autonomous LTR retroelement that has attained the highest copy number of any reported retro-element in O. sativa. Targeting condensed chromatin may be the reason for its success in the small rice genome.

P159 Mutator in the Grasses
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The Mutator system in maize is regulated by MuDR, which carries two genes, mudrA and mudrB. The mudrA gene encodes the putative transposase. DNA gel blot and sequence analysis reveal that mudrA is present in most of the major grass subfamilies. These mudrA sequences can be subdivided into distinct subgroups, or classes, based on phylogenetic analysis. More than one such class can coexist within a single genome. The distribution of these sequences does not match the phylogeny of the grasses in which they are found. Some species closely related to maize, such as sorghum, have only distant relatives of mudrA, and more distantly related species can have more closely related mudrA sequences. The observed distribution of sequences is most likely due to the periodic loss of some classes of mudrA sequences and the proliferation of others. Alternatively, horizontal transfer of sequences between lineages may have occurred. The conservation of key amino acids within and between classes suggests that functional mudrA sequences have undergone repeated "speciation" events throughout their long evolution, and that some of these variants have been more successful than others in specific hosts, either due to stochastic losses, or selection for one variant over others. In contrast to mudrA, mudrB is much less widely distributed. Although the specific function of this gene is not known, it is required for germinal duplication of Mu elements in maize. We have been unable to detect this gene outside of Andropogoneae, either because it is simply missing from MuDR elements outside this tribe, or because it has diverged more quickly than has mudrA. Data base searches reveal that there are MuDR-like elements that lack mudrB in maize, sorghum, rice, and Arabidopis , suggesting that this gene is not an obligate component of all MuDR-like elements. The question then remains, why is mudrB in MuDR and where did it come from?

P160 Transposon Display and Mapping Reveal Clusters of the MITE Hb2 in the Maize Genome
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Miniature inverted repeats transposable elements (MITEs) are a group of non-autonomous transposons characterized by their high copy numbers. They are found in flowering plants, insects, fishes and mammals. Hb2 is a family of MITEs originally discovered as an insertion in the 5, untranslated region of the waxy gene in maize. There are between 12,000-16,000 highly homologous (~90%) copies of Hb2 in the maize genome. Last year, we reported the use of transposon display to generate 565 polymorphic Hb2-derived bands, 50% of which were assigned to chromosomes 1 and 8. Additional genetic mapping indicates that these insertions are not evenly distributed along each of the 10 maize chromosomes, but are found in clusters. Preliminary analysis of the flanking regions suggests that these clusters are associated with other repetitive elements. The molecular mechanisms and evolutionary significance of this distribution will be discussed.

P161 Transposon Display as a method of retrieving sequence from Abnormal chromosome 10
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The Abnormal chromosome 10 (Ab10) of maize results in neocentromere activity of knobs, increased recombination of structural heterozygotes and meiotic drive of all knobbed chromosomes when present in the genome. The genes responsible for these phenomena are located within the large additional segments of chromatin possessed by Ab10. The Abnormal 10 chromosome contains at least one translocation and one inversion of chromatin from Normal chromosome 10 (N10), a differential segment of unknown function, a large heterochromatic knob, and a distal tip of euchromatin. In order to analyze the composition of Ab10 we have employed Transposon Display (TD) of Miniature Inverted-repeat Transposable Elements (MITEs) using a series of deficiencies of the Ab10 chromosome. The shortest deficiency, Df(L), is missing only the distal tip, and has extremely reduced meiotic drive; the most severe deficiency, Df(C), is missing nearly all of the additional chromatin possessed by Ab10. By using TD to compare Ab10, Df(C), and Df(L) to their respective N10 sibs as well as to each other, we have, at present, been able to identify three MITE markers specific to the distal tip of Ab10. Additionally, the cloning of these markers has allowed us to obtain DNA sequence from the distal tip that flanks these markers, which we are in the process of analyzing.

P162 Development and applications of Transposon Display (TD) for several MITE families from rice.
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The genus Oryza contains around 23 different species from Asia, Africa, Australia and Central and South America, two of these are cultivated for food consumption and 7 are allopolyploid. MITEs are estimated to make up 6% of the Oryza sativa genome where they are organized into at least 14 distinct families. Published sequences of the MITE families Explorer, Castaway, Tourist, Olo, Wanderer, Ditto, Snabo-4 and Gaijin served as queries to search the Clemson BAC end database. Primers for transposon display (TD) analysis were designed to recognize conserved regions of the aligned sequences. TD was successfully applied to all MITE families. Comparison of TD profiles revealed that different MITEs showed characteristic levels of insertion site polymorphism (18-56%) between indica (IR64) and japonica (Azucena), thus providing evidence for temporally distinct rounds of amplification throughout the genome. All MITE TD primers were used successfully to amplify MITEs from 9 Oryza species thus providing tools to address the evolutionary influence of MITEs in the genus Oryza. The genomic positions of 40 elements each from the Olo and Tourist families were determined in an IR64 x Azucena doubled haploid mapping population and found to be distributed over all 12 chromosomes. The fact that Oryza is comprised of wild and cultivated species, that the genome of O. sativa will be completely sequenced in the near future and that several MITE families are described makes the genus Oryza a suitable system to study the evolutionary dynamics of MITEs and determine how MITEs contribute to genome evolution in Oryza.

P163 ANALYSIS OF THE CIS-ELEMENTS REQUIRED FOR TRANSPOSON-INDUCED RECOMBINATION IN PLANTS

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Maize Ac/Ds transposable elements can induce homologous recombination between two 5.2-kbp direct repeat sequences that flank the maize p1 gene (Athma, and Peterson, 1991, Genetics 128, 163-173; Xiao, et al., 2000, Genetics 156: 2007-2017). In order to identify the cis-requirements for transposon-induced recombination, we developed an Arabidopsis model system that parallels the maize p1 locus structure. In this system, two partially-deleted GUS reporter gene segments are separated by an intervening sequence containing a Ds element. The addition of Ac transposase induces a 1,000 fold increase in somatic recombination frequency (Xiao and Peterson, 2000, MGG 263, 22-29), detected as GUS+ (blue) sectors. To determine whether recombination requires Ds excision, we tested the effects of two mutations in Ds. One mutant has a deletion of the last base pair at the 3' terminal inverted repeat of Ds; this causes a 3800 fold reduction in Ac excision from the maize p1 locus. A second mutant has a five base pair deletion in the 5' terminal inverted repeat of Ds; this was reported to abolish transposition in tomato. We tested the effects of each mutation on the ability of Ds to stimulate homologous recombination in Arabidopsis. Recombination was greatly reduced, but not abolished, in both cases. These results suggest that transposon-induced recombination may occur even in the absence of transposon excision, albeit at a lower frequency. Possibly, transposase proteins and the sequences with which they interact may provide the basis for development of homologous recombination techniques in plants.

P164 Non-linear Ac/Ds Transposition and Maize Genome Reorganization

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Transposable elements have long been considered as potential agents of large-scale genome reorganization. In maize, particular configurations of transposon termini can induce chromosome rearrangements at high frequencies. We analyzed several genomic rearrangements derived from an unstable allele of the maize p1 (pericarp color) gene carrying both a full-length Ac (Activator) transposable element and a Ac terminal fragment termed fAc (fractured Ac). In one case, a classical maize ear twinned sector yielded two rearranged chromosomes; one contained a large inverted duplication, and the other contained a corresponding deficiency. The rearrangement breakpoints have target site duplications and a transposon footprint, thereby proving that the duplication and deletion chromosomes were generated by a single transposition event involving Ac and fAc termini located on sister chromatids (Zhang and Peterson, 1999. Genetics 153, 1403-1410). Because the transposition process we describe involves transposon ends located on different DNA molecules, it is termed non-linear transposition (NLT). Non-linear transposition can rapidly break and rejoin chromosomes, and thus could have played an important role in generating structural heterogeneity during genome evolution. Nine additional NLT-induced large deletions were analyzed. The deletions are all anchored at the Ac/fAc insertion, and extend to various endpoints up to 4.6 cM proximal of the P locus. We conclude that NLT events can efficiently generate interstitial deletions, and that the resulting nested deletions are potentially useful for dissection of local intergenic regions, and for rapid correlation of genetic and physical maps. Finally, a modified NLT model can explain the origin of several complex maize chromosome rearrangements isolated by McClintock.

P165 Transposons as Genetic Tools for Functional Analysis of Promoter Function *In Vivo*
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The promoter of the maize *A1* flavonoid biosynthetic gene has a modular structure conserved in the *cis*-regulatory regions of other genes in the pathway. In the kernel, *A1* is activated by P (Myb) in the pericarp, and by the C1 (Myb) and R (bHLH) factors in the aleurone for the production of phlobaphene and anthocyanin pigments, respectively. In transient expression experiments, activation of *A1*by P and C1+R involves the proximal P high-affinity sites (^{ha}PBS) or the distal P low-affinity sites(^{la}PBS). The ARE element, a conserved sequence found in other anthocyanin biosynthetic genes, is located between the ^{ha}PBS and ^{la}PBS. Yet, the biological significance of the ARE elements with respect to the transcriptional regulation of these promoters is not well established. The availability of maize *A1* alleles carrying either the *Suppressor-Mutator* (*Spm*) (*a1-m2*) or Mutator (*Mu*) (*a1-mum2*) transposons in the ARE provide great genetic tools for dissecting the *A1* promoter function *in vivo*. We investigated the effect of these two transposable element insertions and of germinal or somatic excisions on the regulation of the *A1* gene by P or R&C1. Our studies suggest a central role of the ARE element on the regulation of *A1* by P and C1&R *in vivo*, function that is not obvious from previous *in vitro* binding or transient expression experiments. Moreover, our studies suggest that mutations in the ARE differentially affect expression of *A1* controlled by P or by C1&R.

P166 Small Transposable Elements isolated from transcripts of the intensifier alleles in1 and In1-D
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Sequence analysis of genomic and cDNA clones of the recessive intensifier1 allele in and the dominant allele Intensifier1-Dilute (In-D) revealed the presence of small transposable elements in the genomic and cDNAs of both alleles. In the intensifier allele In1-D, a 122 bp transposable element called BEB is present in exon 6 of this allele. BEB is also found in transcripts of In-D and leads to non-functional proteins. The BEB element has also been found in a seedling cDNA isolated from a W22 color converted line (Line C). The insert is absent in an EST clone isolated from a tassel cDNA library of line OH43, indicating an insertion event in the Line C cDNA clone. A 315 bp transposable element called ROH was found in the misspliced transcripts of the recessive allele in. The ROH element is integrated at the 3´end of the second intron of in and was also found in truncated transcripts of in. The termination of in mRNAs in the second intron might be due to a poly-adenylation site in the ROH element. So far no other sequence homologies to the ROH and BEB elements have been found in the reported maize genome.

P167 A Targeted Reverse Genetics Approach for Novel Genes and the Study of *Ds* Insertion Patterns in the 140-kb *a1-sh2* Interval

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The *Ac*/*Ds* transposon system was used in a reverse genetics approach to identify the functions of two novel genes in the *a1-sh2* interval: *x1* and *yz1*. To enrich for insertions within the novel genes, the *a1-m4* allele that contains a *Ds1*-like insertion in exon 4 was used to generate a "mini-gene machine". *Ds* transpositions were effectively targeted to the *a1-sh2* interval because the *Ac*/*Ds* transposon family preferentially jumps to genetically linked sites (Greenblatt, 1984). *Ds*-excision events from *a1*, a gene necessary for kernel pigmentation, were evidenced by colored revertant sectors in the aleurone. Approximately 900 plants grown from spotted kernels were screened for *Ds* insertion events in *x1* and *yz1*. Thus far, several unique insertions have been identified in *yz1* while no insertions have been found in *x1*. These results could be due to either gametic selection against *x1* insertions or to a nonrandom pattern of insertion site selection. The patterns of *Ds* transposition within the 140-kb interval are being explored with a particular emphasis on defining those chromosomal features that influence *Ds* insertion site selection.

P168 Isolation of a novel autonomous class 2 (DNA) element with strong target site preference and MITE family members

Zhang, Xiaoyu(1); Jiang, Ning(1); Zhang, Qiang(3); Eggleston, William, B.(2); Feschotte , Cedric (1); Wessler, Susan, R.(1) (1) University of Georgia, Athen, GA 30502, USA; (2) Virginia Commonwealth University, Richmond, VA 23284, USA; (3) Monsanto Mystic Research, Mystic, CT 06355, USA

see TALK ABSTRACT #T6

SESSSION 2

THE GENOME

FRIDAY MORNING

LATE SUBMISSIONS (4)

P169 Isolation and identification of genes expressed differentially in rice inflorescence meristem with suppression subtractive hybridization

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A subtracted cDNA library of rice (Oryza sariva L.) inflorescence meristem (1M) was constructed using the suppression subtractive hybridization (SSH) method. The cDNAs of the rice shoot apical meristem (SAM) were used as "driver"? and inflorescence meristem (IM) as "tester"? in the experiment, respectively. Forty of 250 randomly chosen cDNA clones were identified by differential screening, which were IM-specific or IM-highly expressed. Most of the rice IM cDNAs cloned by SSH appear to represent rare transcripts, 40% of which were derived from truly differentially expressed genes. Of all the forty sequenced cDNA inserts, eleven contain the regions with 60%-90% identity to their homology in GenBank, eighteen are expected to be new genes, only two correspond to published rice genes.

P170 Cloning and characterization of two cDNAs encoding rice MADS box Protein

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To elucidate the relationship between MADS box gene and rice morphogenesis, RT-PCR with MADS domain specific primers was performed to isolate MADS box gene form young panicle of rice "Zhenshan 97B". Two cDNAs, designated as nmads1 and nmads3, displayed the structure of a typical plant MADS box gene which consists of the MADS domain, I region, K domain, and C-terminal region. Based on sequence homology, nmads1 is classified as a member of GLO subfamily, and nmads3 belongs to AGL2 subfamily. Hybridization analysis revealed that nmads1 and nmads3 were preferentially expressed in rice redifferentiated callus and young panicles but were not in ricer seedling. An additional transcript of nmads1 was also found in young panicle of cytoplasmic male-sterile line Zhenshan 97A but was not in its maintenance line Zhanshan97B.

P171 Differential display of mRNA between hybrid F1 and its parental inbred lines in maize (Zea mays L.)

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Heterosis is a universal biological phenomenon in living things. It has been utilized extensively in crop breeding, the improvement of fish and the breed of domestic animal for a long time. Great success has been achieved in practical production of the utilization of heterosis, but in theory, we have known little about the mechanism of heterosis. At the beginning of this century, Bruce et al. proposed the dominance hypothesis to explain the cause of heterosis development, and then Shull et al. Postulated the superdominance to clarify the hereditary basis of heterosis. These hypotheses were only theoretical models; no experiment has been made to demonstrate it till now. Recently, Peng and Pardee developed and approach, named mRNA differential display, to distinguish mRNA in comparative studies. In search of the mechanism of heterosis development, we will use this method to analyze the differential expression of genes between hybrid F1 and its parents. By means of comparison between gene expression products of hybrid F1 and that of its parents, we will understand how genes express in different hereditary backgrounds (hybrid F1 or its parents), and what genes relate to the formation of heterosis. On the basis of the knowledge, we will explore the relationship between heterosis and the structure and constituent of plant genome, and then verify the cause of resulting in heterosis in hereditary essence.
We have analyzed the difference of the level of gene expression between hybrid maize F1 and its inbred parents with mRNA differential display, and then unraveled a more important phenomenon that has been known little before. We have established a novel strategy for studying the molecular basis of heterosis development in this work.

P172 Alteration of gene expression in rice hybrid F1 and its parental seedlings

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Hybrid rice (Oryza sativa L.) seedling is more vigorous in root development and plant growth than its parental lines in the tested indica rice of hybridized combination (Shanyou63 (F1): female Zhenshan 97a x male Minghui63). Analysis of the difference in gene expression between the hybrid F1 and its parental seedlings by means of mRNA differential display indicated that gene expression of the parental lines was obviously altered the hybrid F1 both in quantity and quality. Quantitatively, there were over-expression and under-expression of genes in hybrid F1 with genetic expression trend forwards a single parent. Qualititatively, hybrid F1 could have specific gene expression, single parent (maternal or paternal) gene silence, co-suppression of paternal genes, and single paternal gene expression. The relationship between heterosis formation and alteration of gene expression of parental lines in hybrid F1 was also discussed.